

*This thesis is dedicated to my dear wife Margaret,
who gave up so much to give me this chance*

The work presented in this thesis is, to the best of my knowledge and belief, original, except as acknowledged in the text. The material has not been submitted, either in whole or in part, for a degree at this or any other University.

David W. Stephens

April 2000

**EFFECTS OF CHANGES IN THE SUPPLIES OF
NITROGEN AND CARBON ON THE PHENOLOGY AND
GROWTH OF *NOTHOFAGUS FUSCA* (HOOK.F.)
OERST.**

**A thesis
submitted in partial fulfilment
of the requirements for the Degree
of
Doctor of Philosophy in Biological Science
in the
University of Canterbury
by
David W. Stephens**

University of Canterbury

2000

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ABSTRACT

The tree genus *Nothofagus* is widespread in the Southern Hemisphere as a characteristic component of the Southern flora. *Nothofagus fusca* (Hook.f.) Oerst. is an important species in many natural forest systems of New Zealand. It is unusual in having an evergreen habit, with a marked deciduous loss of old leaves in spring as new leaves emerge and expand, so that the tree is never devoid of leaves. This dual strategy has implications for processes of uptake and allocation of nitrogen (N) and carbon (C) to new growth in spring, which have not been investigated before. Evergreen trees store N in leaves during winter, and remobilisation to new growth in spring is independent of leaf senescence. Winter-deciduous trees remobilise N from storage in stems or roots. Responses to manipulations of N and C supplies to young trees of this species were studied in order to characterise, and understand, this unusual phenology.

Measurements of stem, bud and leaf growth, leaf loss, and photosynthetic characteristics of leaves were made on young trees, grown in sand while irrigated with high (HN, 6 mM), medium (MN, 3 mM) and low (LN, 0.5 mM) concentrations of nitrogen, during two successive annual growth cycles. There were differences between treatments in the nitrogen content per leaf on a mass and an area basis, and specific leaf area, in the first cycle, but not during the second cycle. During the first cycle, an initial adjustment to increased N supply resulted in larger average leaf size as well as more leaves. At the start of the second cycle the number and mass of buds had increased approximately 4 times in MN trees, and approximately 8 times in HN trees, relative to LN trees. Comparative increases in leaf number measured at the end of spring, and at autumn, of the second cycle were 6-fold, and 19- and 22-fold. However, there was no change in average leaf size, maximum rates of

photosynthesis or values for photosynthetic parameters. Leaf loss from the canopy during late summer and mid-autumn increased with nitrogen supply. This was explained as a mechanism to avoid summer water deficit, and support for this was provided from measurements of the carbon isotope ratios of the leaves.

The effect of nitrogen (N) supply on biomass, and N storage and remobilisation for spring growth was also investigated in the same young trees, during the same two annual growth cycles. N acquired during the first cycle was labelled with ^{15}N enriched to 5.5 atom % excess. By mid-autumn of the second cycle, dry weights of whole tree, stem, total leaves and roots were over 10-fold greater in HN and MN trees. N was stored in roots and the quantity stored was 20-fold greater in MN trees ($P < 0.001$). Stored N was remobilised into new leaves and stem extension during spring, comprising approximately 40% of all N in those tissues. HN and MN trees continued N uptake during winter dormancy. The amounts approximated half of all labelled N acquired during the first cycle, showing the importance of winter uptake in this temperate species. N remobilisation from roots may have been accompanied by fine root turnover. The number and mass of overwintering buds set during the first cycle was significantly greater in HN trees. It is possible that the absence of significant growth differences between HN and MN trees was due to the HN supply stimulating foliage growth beyond an optimum. Root storage has implications for understorey competition because of the effect on sub-soil spatial interactions of N and water availabilities.

Ammonium nitrate fertiliser enriched to 10 atom % was applied to juvenile trees of *Nothofagus fusca* grown for 5 years at ambient and elevated CO_2 concentrations, about 2.5 weeks before budburst. The aim was to determine the timing of root N-uptake relative to budburst. Bud samples and, (following

budburst), leaf samples, were harvested three times per week until all leaves on each tree were fully expanded. There were no significant differences between treatments in the timing of onset of budburst, or its duration, or in the onset of loss of leaves from the previous season. This was notwithstanding the greater mass of buds on trees growing at ambient CO₂ concentration. There was a trend for trees growing at elevated CO₂ concentration to retain 30% of old season's leaves beyond 70 days from the start of spring. There was no difference in the absolute rate of expansion of new leaves but the seasonal increase in the mass of individual leaves was delayed in trees growing at elevated CO₂ concentration. Individual leaves of trees growing at elevated CO₂ concentration were greater in area and mass. Enhancement of leaf area was probably not due to any interactive effect of C supply on tree N status. Nitrogen concentration of leaves, on a mass basis, N_m , was lower in trees growing at elevated CO₂ concentration. Growth of these trees at elevated CO₂ concentration for five years did not produce changes in the onset of budburst or leaf loss during spring-early summer. Nor were there changes in the pattern of simultaneous root N-uptake, seen in younger trees.

Conclusions are drawn as to why *N. fusca* is unusual in obtaining benefits from both evergreen and deciduous habits. The phenology of leaf growth and leaf loss in this species is unique. It not only provides deciduous advantages for storage and remobilisation of N in roots, but also evergreen capabilities of N-remobilisation independently of senescence, and root N-uptake during winter. The response to increased N availability is to expand the canopy by an increase in leaf numbers and associated stems. This renders the tree vulnerable to occasional summer drought. The presence of a strategy that enables leaves to be shed to avoid this does not compromise the need to store N during winter, since storage is in roots.

CHAPTER 1

INTRODUCTION AND RATIONALE

1.1 INTRODUCTION

Since the advent of concern about the global implications of a predicted increase in the concentration of atmospheric carbon dioxide (CO₂) many experiments have been conducted to test the response of plants to changes in carbon supply. A considerable number of these experiments have been devoted to tree species because of their important role in wood formation, and hence carbon storage (see Jarvis, 1995; Ceulemans, Janssens & Jach, 1999). There is now a corresponding interest in the need to reconcile results from these studies (eg Curtis, 1996; Curtis & Wang, 1998), and in particular to elucidate the crucial role of nitrogen (N) supply in mediating the effects of an increased carbon (C) supply (e.g. Stitt & Krapp, 1999; Geiger *et al.* 1999). There is also a need to characterise responses to changes in C supply that might influence competitive interactions both within, and between, species. Because trees take up C from the atmosphere through the action of leaves, the ability to survive and compete can depend primarily on spatial or temporal separation of individual canopies (Begon, Harper & Townsend, 1990; Kupperts, 1993). Particular requirements and mechanisms for separation may alter as any individual tree grows from seedling stage to maturity, consequent on changes in the relationships with competitors or with other environmental factors.

In temperate regions seasons are markedly cyclic and periods of tree growth generally coincide, (also cyclically), with conditions that are favourable for growth (Richards, 1996). Trees are generally dormant at other times of the year. As part of this process, transitions and periodicities are demarcated by rhythmic biological

events (phenologies), such as budburst, stem extension, leaf expansion, changes in total leaf area, and pulsed leaf loss. These events not only define the time and space for nutrient uptake (and hence quantities) by any individual tree, they also influence temporal and spatial separations of nutrient uptake between competitors. More study of the impacts of changes in carbon supply on processes of leaf area development is required (see Ceulemans & Mousseau, 1995). For example, changed phenology resulting in an earlier or later development of leaf area in spring might result in changes to the species composition of forests due to adjustments in competitive advantage or disadvantage (see Saxe, Ellsworth & Heath, 1998).

The purpose of the investigations described in this thesis was to test the effects of changes in nitrogen and carbon supply on the phenology and growth of *Nothofagus fusca* (Hook.F.) Oerst. (Fagaceae), a large forest tree that is endemic to New Zealand.

1.2 BIOLOGY AND PHENOLOGY OF *NOTHOFAGUS FUSCA*

1.2.1 The genus

The genus *Nothofagus* is widespread throughout the Southern Hemisphere. It comprises about 37 species (Setoguchi *et al.*, 1997) of broadleaf trees, currently distributed as important components of extensive tracts of native forests in South America (temperate Argentina and Chile), Australia, New Zealand, New Guinea and New Caledonia (Boland *et al.*, 1984; Wardle, 1984; Poole, 1987; Veblen, Hill & Read, 1996; Setoguchi *et al.*, 1997). It has been estimated that, within New Zealand, *Nothofagus* ecosystems occupy more than 2×10^6 ha. and comprise over 30% of carbon (C) storage in native forests (Hollinger & Hunt, 1992). Past systematic analysis has placed importance on differences of leaf-fall habit (deciduous, semi-

deciduous, or evergreen) shown between *Nothofagus* species (van Steenis, 1953). The genus therefore contains examples of differences between species, in cyclic patterns of canopy growth and replacement.

1.2.2 Cyclic pattern of growth and budburst in New Zealand species

All four endemic New Zealand species (*N. fusca*, *N. truncata*, *N. solandri* vars. *solandri* and *cliffortioides*, and *N. menziesii*) are inactive during winter, and begin growing in spring-early summer. Budburst is from buds that contain leaf and floral primordia set in the previous autumn (Wardle, 1982). They are also monoecious, with female flowers borne distally from male flowers on the same leafy shoot that develops following budburst (Poole, 1950). If floral primordia are present, the male flowers emerge first from the bud. All New Zealand *Nothofagus* species are possibly self-sterile (Wardle, 1984) and minute female flowers appear above the male flowers generally only after all pollen has been shed (Poole, 1987). Stem extension and leafy shoot development then continues above the floral region (Wardle, 1984). If floral primordia are not present, budburst precedes the emergence and development of new stem and leaves.

1.2.3 Cyclic pattern of leaf fall in New Zealand species

All New Zealand species are evergreen in the sense of maintaining a canopy of leaves throughout the year, but exhibit to a varying extent deciduous seasonal pulses of leaf loss around spring (Wardle, 1984). The latter characteristic is most pronounced in *N. fusca*. Mature trees commence shedding leaves at budburst during the transition to new season's canopy (Russell, 1936) and practically all leaves

remaining from the previous growing season are shed during this process (Bussell, 1968). So, while all old leaves might be lost, the tree is never bare of leaves.

1.2.4 Cyclic production of stems and leaves in *N. fusca*

The patterns of cyclic growth of new stem and leaves in individuals or cohorts of *N. fusca* appear to differ depending on age. Bussell (1968) observed that seasonal leaf production and stem extension of *N. fusca* seedlings may be for a longer period than for mature trees. In first year seedlings, it was continuous, and synchronous among all plants, until resting buds were formed in autumn. In two to four year old seedlings, there were two or three asynchronous growth flushes, with intervening quiescent periods sometimes accompanied by formation of intermediate resting buds, with final resting bud development in autumn. In young trees, about 10 years old, there were two distinct synchronous flushes, one in spring, and the other in autumn, when resting buds were formed. In mature trees, there was a single flush, followed early in the season by formation of resting buds.

1.2.5 Distribution of endemic *Nothofagus* along elevation and precipitation gradients

Ogden, Stewart & Allen (1996) have plotted the distribution of endemic *Nothofagus* species along elevation and annual precipitation gradients in the South Island (Fig. 1.1). Based on this data, the extreme elevation range of the genus is from sea level (*N. menziesii*) to c.1380 m.a.s.l. (*N. solandri* var. *cliffortioides*), and the extreme precipitation range is c.420 mm to c.9200 mm (*N. menziesii*, in both cases). *N. fusca* is intermediate within this overall distribution, c.220-900 m.a.s.l. and c.1420-7000 mm, respectively.

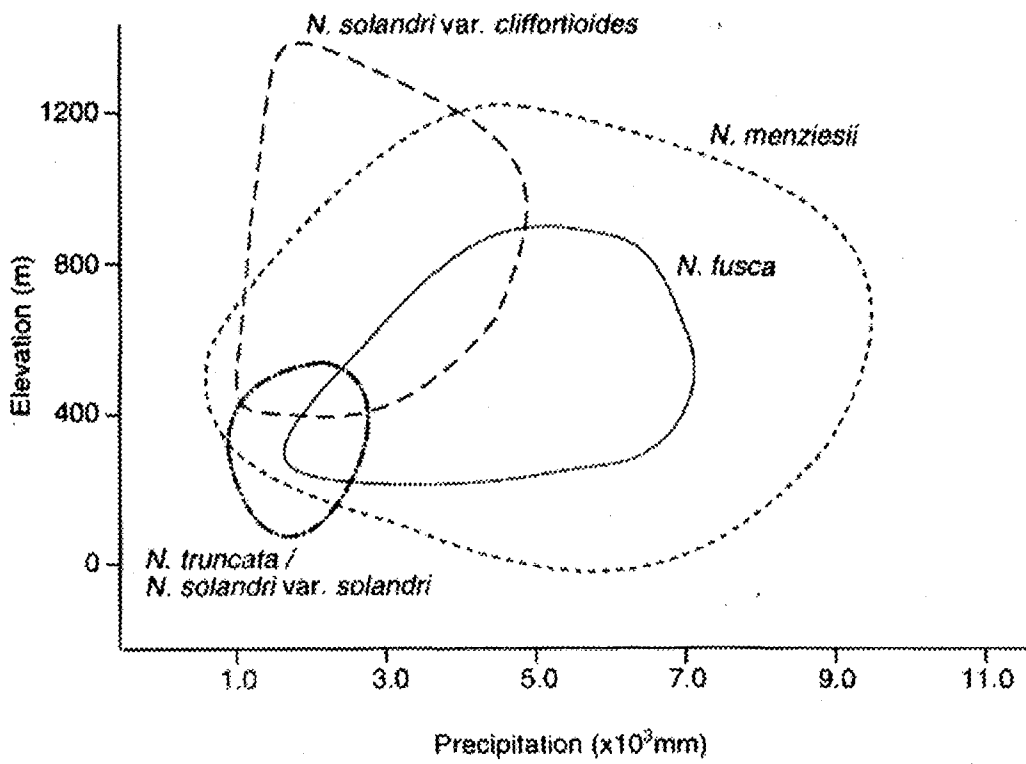


Figure 1.1 Distribution of endemic *Nothofagus* spp. along elevation and precipitation gradients in the South Island. (Reproduced from Ogden, Stewart & Allen, 1996)

Despite a normal annual rainfall of c.1420 to 7000 mm, *N. fusca* must nevertheless endure severe droughts with a return period of 30 years predicted for both main islands (North Island: Grant, 1984), (South Island: Jane, 1985). During these drought periods, precipitation over the period from spring to the following winter may be as low as c.220-680 mm, and trees may experience dieback up to the timberline, e.g. 1470 m.a.s.l. (Grant, 1984). Twelve-month droughts (460-630 mm per annum) may recur every 60 years (Grant, 1984; Jane & Green, 1983). *N. fusca* may have a maximum age of 450-600 years (Wardle, 1984), in which case trees may have endured severe spring-winter drought, and severe annual drought on no fewer than 15 and 7 occasions, respectively.

1.3 PHENOLOGY IN TREES

1.3.1 Definition of phenology

The term “phenology” refers strictly to the study of periodic biological events but, in practice, it is applied to the periodic phenomena themselves (Begon, Harper & Townsend, 1990). A useful definition has been provided by Leith (1970), who used the term to describe the art of observing life cycle phases or activities of plants, in their temporal occurrence throughout the year. Pursuant to this concept, a phenological calendar (as opposed to the astronomic, or civil, calendar) can be constructed showing seasons marked, not by calendar dates, but rather by dated groups of phenological events. These include seasonal fluctuations in productivity and consumption and also:

- (a) all life cycle phases (phenophases), including germination, budburst, leaf development, flowering time, fruiting time, fruit or seed dispersal, plant or leaf death, and litterfall

- (b) phenometric analyses, including leaf area index, chlorophyll content, photosynthesis activity, respiration rates, organic productivity, and energy and nutrient consumption.

1.3.2 Ecological implications

Phenology influences two broad ecological consequences in terms of the competitiveness and survival of trees. The first of these is the achievement of an efficient balance between tree growth and resource uptake. This is largely determined by the synchronisation of periods of growth and dormancy with appropriate seasonal phases of temperature and day length cycles (see Saxe, Elsworth & Heath, 1998), and is perhaps best illustrated by winter-deciduous Northern Hemisphere species which are entirely bare of leaves during winter. The second, the process of niche differentiation (see Begon, Harper & Townsend, 1990), forms the basis for co-existence of competitors, and is largely determined by differences between species and individuals or cohorts in the timing and quantities of resource utilisation (e.g. Kupperts, 1993; Gill, Amthor & Bormann, 1998). For example, Kupperts (1993) investigated succession in a dense hedgerow site in Northern Bavaria containing a large number of competitive winter-deciduous woody plants. He reported that two components influenced the competitive strength of species that dominated in different stages of succession. The first was biomass increment, and the second, the architectonic arrangement of plant matter in space. In terms of this analysis, species distribution appeared to be the result of the combination of physiological and morphological factors separating pioneer niches and niches of later successional species. In another example, Gill, Amthor & Bormann (1998) quantified the leaf phenologies of saplings and overstorey trees of

Acer saccharum, *Fagus grandifolia*, and *Viburnum alnifolium* in a North American hardwood forest. They reported that leaf expansion occurred earlier in spring, and green leaves were retained later in the autumn, in saplings and shrubs, than in overstorey trees. This appeared to permit long-term persistence during juvenile phases, of late successional species in the shrub layer of mature forest.

1.3.3 Impact of C and N supplies on phenology

Like other growth processes, phenology may be affected by nutrient supply, uptake, and plant nutrient status, including C availability (see Jarvis, 1995; Ceulemans & Mousseau, 1994; Saxe, Ellsworth & Heath, 1998) and the interaction between C and N supplies. Generally, in combination with an elevated C supply, low N supply causes little change in above-ground growth but leads to stimulation of root growth, whereas high N supply stimulates above-ground growth (Jarvis 1995). The study of Silvola & Ahlholm (1993) provides an elegant illustration of the interactive effect of C and N supplies on shoot length growth rhythm of *Salix phylicifolia* cultivated at four different levels of both C and N supply. A high C/N supply ratio led to a shorter growing season, and a low ratio to a longer one. The variation was as great as 30%.

A number of recent reviews have listed the phenologies which may be affected potentially by an enhanced C supply (see Ceulmans & Mousseau, 1994; Jarvis, 1995; Saxe, Ellsworth & Heath, 1998). These include the onset of budburst, the weight of buds at budburst, the rate of new leaf expansion, the number and area of individual leaves in the canopy, the onset of leaf senescence and abscission, and leaf longevity. Physiological characteristics that are influenced by C supply include the photosynthetic capacity of individual leaves, leaf nitrogen concentration (both mass-

and area-based), and specific leaf area (the ratio between leaf area and leaf weight) (see Cotrufo, Ineson & Scott, 1998; Medlyn *et al.* 1999).

1.3.4 The importance of understanding N relations

The interactive effect of C and N supplies, where N supply is sub-optimal, is to produce a reduction in plant N status, resulting in nitrogen limitation (Geiger *et al.*, 1999). The effect seen in *Nicotiana tabacum*, when investigated by Geiger *et al.* (1999), was quite striking at intermediate levels of N supply compared with super-optimal supply (10 mM ammonium nitrate, or 20 mM nitrate). The reduced N status was considered to be just sufficient to avoid N limitation in ambient CO₂ concentrations. However, while it was quantified for *Nicotiana tabacum* in that study, it has not yet been quantified for any forest tree. Therefore it is necessary to an understanding of C effects on phenology and physiology in any tree species, to appreciate also how patterns and mechanisms of N utilisation are affected by tree N status, at different levels of N supply.

The influence of N on the relationship with C supply, and on tree phenology, can be mediated, in perennial species, by both root uptake of N from the soil and remobilisation of N from storage (Millard, 1996). The level of N supply available for new growth from these sources can influence phenological and physiological changes in the canopy that consequently affect the timing and uptake of carbon. Increased total photosynthesis in response to an elevated N supply may result from one or more of the following factors (see Brix & Ebell, 1969):

- (a) an enhancement in the photosynthetic capacity of individual leaves
- (b) an increase in leaf numbers producing an enlargement of the total photosynthetic area of the whole canopy

- (c) an increase in the area of individual leaves also producing an enlargement of the total photosynthetic area of the whole canopy.

How changes in total canopy area are achieved may also depend on N availability affecting whether leaf and stem extension is determinate, or indeterminate on a species-specific basis (see Puntieri *et al.*, 1998).

1.4 RATIONALE

The inter-related effect of both N- and C-supplies in influencing plant growth has been recognised in a number of recent meta-analyses that synthesise the results of many preceding studies (e.g. Curtis, 1996; Curtis & Wang, 1997). These have shown that C effects on growth can be expected to result only where N is not in short supply. An evaluation of the comparative importance of C- and N-supplies, respectively, in bringing about this response, must depend on the adequacy of criteria used to assess whether tree N-status has been significantly affected by C supply (Stitt & Krapp, 1999). It has been reported recently that, at low levels of N supply, an enhanced C supply does not result in an increased growth of biomass, because it exacerbates an N limitation (Geiger *et al.*, 1999). One question, important to this study, was:

Is it N supply, or is it C supply, which is important to phenological and physiological changes in the canopy (i.e. the plant organ responsible for C uptake) of N. fusca?

Nothofagus fusca was chosen for this study because it is an important native forest species which, (compared with other endemic *Nothofagus* species), tends to occupy

more fertile sites (Ogden, Stewart & Allen, 1996) and so might be particularly sensitive to changes in N status. Also it is an evergreen species with a marked deciduous habit of leaf loss in spring, and the dynamics of N supply and utilisation has not been investigated previously in this unusual class of tree.

Juvenile trees of *N. fusca* have been grown at elevated concentrations of CO₂ in open-top chambers at Bromley, Christchurch, for five years (Whitehead *et al.*, 1995). This afforded a unique opportunity to study any changes in phenology that might have developed as a consequence of long-term growth in an atmosphere of elevated CO₂ concentration. In particular, any changes in phenology occurring between seedling and juvenile stages (Bussell, 1986), would have done so at the enhanced C supply level.

1.5 A CONCEPTUAL MODEL

Reported studies have shown that, in general, autumn-deciduous trees first withdraw N from senescing leaves into perennial woody tissues, for winter storage. In a subsequent process, storage N is then remobilised into new seasonal growth in spring (e.g. *Malus domestica*, Millard & Thomson, 1989; Millard & Neilsen, 1989; Nielsen *et al.*, 1997; *Prunus* spp., Taylor & May, 1967; Weinbaum *et al.*, 1978; Weinbaum *et al.*, 1984; Weinbaum *et al.*, 1987; *Juglans regis*, Deng, Weinbaum & De Jong, 1989; Weinbaum & van Kessel, 1998; *Betula pendula*, (Wendler & Millard, 1996), and *Acer pseudoplatanoides*, (Millard & Proe, 1991) and *Fraxinus excelsior*, (Marmann *et al.*, 1997). In contrast, evergreen species store N during winter, primarily in leaves or needles, for remobilisation in spring (e.g. *Citrus* spp., Legaz *et al.*, 1982; Legaz, Serna & Primo-Millo, 1995; *Eucalyptus globulus*, Wendler *et al.*, 1995; and *Picea sitchensis*, Millard & Proe, 1993). This process is independent of senescence.

Because *N. fusca* sheds its old leaves about the time of budburst and the growth of new leaves it seemed reasonable to hypothesise that N would be remobilised from old leaves for new leaf and stem growth. This would comprise a singular event during spring and early summer, without the intermediate retranslocation to a winter storage site in stem or roots, seen in winter-deciduous species. Any effects of N or C supply on C acquisition could be determined, however, by reference to the entire canopy of new leaves. The model from which this study proceeded is shown in Fig. 1.2.

Key features to note from the model proposed in Fig. 1.2 are:

- (i) When the tree breaks dormancy in the spring of Year 1, it does so with two resources to meet spring growth demand for C and N. The extant canopy of leaves (the first resource) contains N in storage, and remobilisation of this N to new growth is supplemented by root uptake from the soil N pool (the second resource). The size of the N storage pool will be determined by the size of the extant canopy. The extant canopy also determines the amount of C uptake during the initial period after budburst while emerging leaves are primarily C sinks. The tree also contains a third resource, over-wintering buds, which determine the potential for demand for N and C, both immediately at budburst, and subsequently, as a consequence of canopy enlargement and associated whole tree growth. They also determine the ultimate size of the new seasonal canopy, and the seasonal uptake of C and nitrogen. Demands driven by stem cambial, and root, meristems are assumed to be subsidiary to bud meristematic demand in this model. In so doing, it is assumed that budburst, expansion of leaves and stem extension will all drive the need for production of additional stem conducting, and root, tissue.

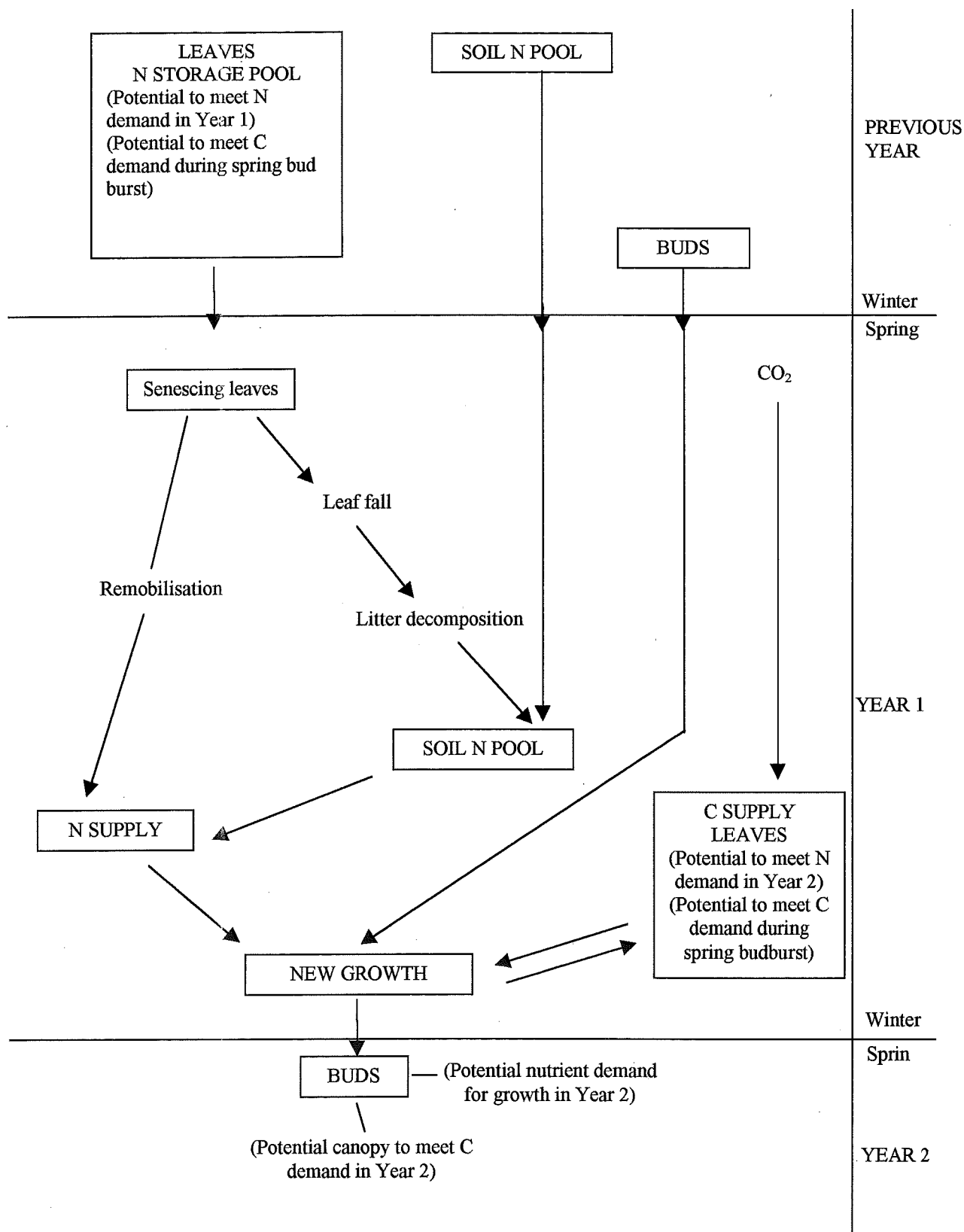


Figure 1.2 Diagram showing components of C and N supply, and interactions with C and N demand for growth in *N. fusca* for two successive seasons.

- (ii) Senescing and abscising leaves are the source of remobilised N, and also contribute to the soil N pool, in Year 1 and successive years.
- (iii) There is an interaction between C supply and the size of the new canopy in Year 1, and this influences the size of the N storage pool, and potential for initial C uptake in Year 2.
- (iv) The quantities of N and C uptake, and their influence on growth in Year 1, affect not only growth during Year 1, but also the number of overwintering buds produced, and hence the size of potential demand, growth, and the uptake of C and N in Year 2.
- (v) Affected phenologies are the timing of budburst, timing of leaf senescence and abscission, total canopy leaf areas (both of old leaves in spring, and of all leaves in Year 1 and Year 2), the size, number and photosynthetic capacity of individual leaves and the extension of new stems.

1.6 OVERVIEW

Analysis and consideration of the general question outlined above required the formulation and investigation of subsidiary questions and issues. These are described in the sections that follow. The topics covered in the following chapters include:

Chapter 2

To determine the effects of an enhanced C supply on canopy characteristics and phenology, it was essential to know first how N supply, alone, might affect those variables. Nitrogen supply can affect C uptake directly by influencing the size and capacity of the canopy. Measurements described in this chapter were carried out on

potted trees that received three different concentrations of N supply for the duration of the experiment. The aim was to determine (i) whether there would be any N-effects on total C uptake due to changes in (a) phenologies of budburst, total and individual leaf areas, and the onset of leaf loss or (b) the physiology and photosynthetic capacity of individual leaves and (ii) the effect that changes in N supply have on overall growth.

Chapter 3

To determine the interacting effects of C and N acquisition on tree phenology and physiology, it is also necessary to understand how, and when, the tree uses N, available both internally and externally, to meet the demand for N for new growth. Changes in phenology, in particular, have the potential to alter the timing of peak seasonal demand for N and so alter processes underlying N uptake and allocation within the plant. Measurements described in this chapter were made on the same potted trees described in Chapter 2. The aim was to characterise (i) the storage site for N within the trees, and the quantity remobilised into new growth in spring-early summer, and (ii) the timing and quantity of root uptake of N allocated to new growth, relative to remobilisation of N from internal storage. Central hypotheses were that storage would be in over-wintering leaves, and that demand for N for new growth would be met initially from stored N.

Chapter 4

Having achieved the results reported in Chapters 2 and 3, the aim of the experiments described in this chapter was to test the effects of an enhanced C supply on (i) the phenologies of budburst and leaf loss, and the expansion rate and size of new leaves, and (ii) the timing of root uptake of N relative to budburst and the early stages of

leaf expansion. If the uptake of N is deferred, it might indicate an initial reliance on internal N storage. Measurements described were made in large open-top chambers, on juvenile trees that had been growing at two levels of C supply for five years.

Chapter 5

The final chapter consists of a general discussion and synthesis of the results. The implications of findings to our understanding of (i) C and N effects on phenology (ii) ecology of *N. fusca*, and (iii) possible responses to environmental change, are discussed. Further questions are asked, and suggestions made as to work which might follow.

CHAPTER 2

EFFECTS OF NITROGEN SUPPLY ON PHENOLOGY AND CARBON ASSIMILATION IN YOUNG TREES OF *NOTHOFAGUS FUSCA* (HOOK. F.) OERST.

2.1 INTRODUCTION

Plants growing under nitrogen limiting conditions generally respond to increased nitrogen supply with increased growth rates and biomass. Using data collected from 50 forest stands in cold-temperate Wisconsin and eastern Minnesota, Reich *et al.*, (1997) established a linear relationship between nitrogen availability (based on the rate of nitrogen mineralisation) and above-ground net primary production, with evergreen conifers and deciduous hardwoods following similar patterns in the regression.

This increased growth performance is supported by an enhancement of whole tree photosynthesis. This can result from morphological changes that increase total leaf (hence photosynthetic) area. It can result from physiological or biochemical changes producing greater photosynthetic performance per unit leaf area. Depending on the species, enhanced growth may be due to a combination of these responses. Increased canopy area may be due to enlarged leaf size (Mitchell & Chandler, 1939; Heilman & Xie, 1994), or increases in total leaf number (Coyne & Van Cleve, 1977), or from both (Brix, 1981a, 1981b; Walters & Reish, 1989; Ibrahim *et al.*, 1997). Increased photosynthetic performance of individual leaves results from increased leaf nitrogen content and nitrogen allocation to increased photosynthetic capacity) (Brix & Ebell, 1969; Peace & Grubb, 1982; Walters & Reich 1989; Reich

et al., 1995). There may also be an increase in specific leaf area, S , (Waring *et al.*, 1985).

Increased photosynthesis per unit leaf mass or area depends on a general relationship between nitrogen content and photosynthetic capacity of individual leaves (Field, 1983; De Jong & Doyle, 1985; Field & Mooney, 1986; DeJong, Day & Johnson, 1989; Evans, 1989; Reich *et al.*, 1995), which, in any species or functional group, is influenced by S (Reich *et al.*, 1998). Using field data collected from 107 species from six different biomes, and literature data for 165 species from diverse sites, Reich *et al.*, (1998) found the relation between photosynthesis at saturating irradiance $A_{\max Q}$, on a mass basis and leaf nitrogen concentration on a mass basis, N_m , was stronger than the same relationship on an area basis. They suggested that the general relationship is made up of a series of nested relationships, with increasing slope as S , and usually leaf nitrogen concentration, increases. Evergreen species with their thicker or denser leaves have lowest values of S , N_m and $A_{\max Q}$, forbs have highest values, and deciduous species (broad-leaved and needle-leaved) have intermediate values. Species with higher S have a higher $A_{\max Q}$ per unit leaf nitrogen, and at any value of S , $A_{\max Q}$ increases with increasing N_m . Intraspecific variation in $A_{\max Q}$ per unit variation in leaf nitrogen is higher in species with highest values of S .

The response of growth to nitrogen supply was investigated for young *Nothofagus fusca* (Hook F.) Oerst trees, one of five *Nothofagus* taxa endemic to New Zealand. In seedlings, saplings and mature trees of this species there is a pronounced pulse of leaf fall after budburst (Bussell, 1968), and (at least for mature trees) all previous season's leaves are shed by the end of the succeeding summer (Russell, 1936). Stem extension and new leaf formation is completed by early

summer, whereas seedling growth is more or less continuous until resting bud formation in autumn (Bussell, 1968). *N. fusca* tends to occupy more fertile sites than the other endemic species (Ogden, Stewart & Allen, 1996), and this suggested that growth, photosynthetic capacity and phenology might be sensitive to variation in soil nitrogen availability.

The objectives of this study were to investigate the seasonal response of leaf nitrogen concentration and the timing and growth of leaf area in the trees in relation to photosynthetic characteristics.

2.2 MATERIALS AND METHODS

2.2.1 Plant material

Seedlings of *Nothofagus fusca* (Hook f.) Oerst, which were approximately 20 mm tall, were collected beneath a closed forest canopy of mature trees near the township of Maruia, South Island, New Zealand (42° 12' S, 172° 15' E) and grown in a nursery for two years. The trees were then transplanted into pots containing 9 litres of medium grade sand, arranged randomly in three rows, each containing 48 trees running east to west at an outdoor nursery. The trees were sheltered from wind and each was enclosed in a fine mesh to ensure that any falling leaves were collected from the surface of the pots. Mean annual rainfall in this area is 550 mm, and the mean maximum/minimum air temperatures in mid-summer (January) and in mid-winter (July) are 21/12 °C, and 11/ 2 °C, respectively. Between September and January, föhn winds commonly result in high temperatures (up to 34 °C and low humidity (< 20 %) for up to several days at a time.

The trees were supplied with three levels of nitrogen by measured irrigation of pots with nutrient solution three times per week. The three concentrations, applied

as ammonium nitrate, were 6 mM (high nitrogen, HN), 3 mM (medium nitrogen, MN) and 0.5 mM (low nitrogen, LN). All other nutrients were as used in Millard & Proe (1993). Care was taken to ensure that soil in pots always remained moist and free-draining, and supplementary water was supplied by sprinkler during dry periods when necessary.

2.2.2 Measurements of biomass and leaf characteristics

The experimental period lasted for 618 days from 1 December 1996 to 10 August 1998, covering two successive annual cycles starting with budburst in spring. The times of harvests, leaf collections and measurements are shown in Fig. 2.1. During 1998, (days 396 to 618 of the second cycle) trees experienced exceptionally dry conditions, with mean annual rainfall of 382 mm., and mid-summer and mid-winter temperatures of 24.5/12 °C, and 12.4/3.4°C, respectively. Six destructive harvests in all were made, two in the last part of the first cycle, in June (day 211) and September (day 283), and four within the second cycle, in October (day 321) and December (day 370) 1997, and in February (day 401) and April (day 501) 1998. The October harvest was timed to coincide with the approximate mid-period of budburst in all treatments. Six trees from each treatment were selected randomly for each harvest. Stem diameter and height were measured and new stem growth was identified using the position of internodes and stem colour. The trees were removed and separated into buds, leaves and branches. Roots were carefully removed from pots in order to retain all material, and washed. Numbers of branches (defined as all supporting structures not being petioles), leaves and buds were counted, and total leaf areas measured using a leaf area meter (model LI-3100, Licor Inc. USA). All tree components were freeze-dried, weighed dry, and ground in a Tema or ball mill.

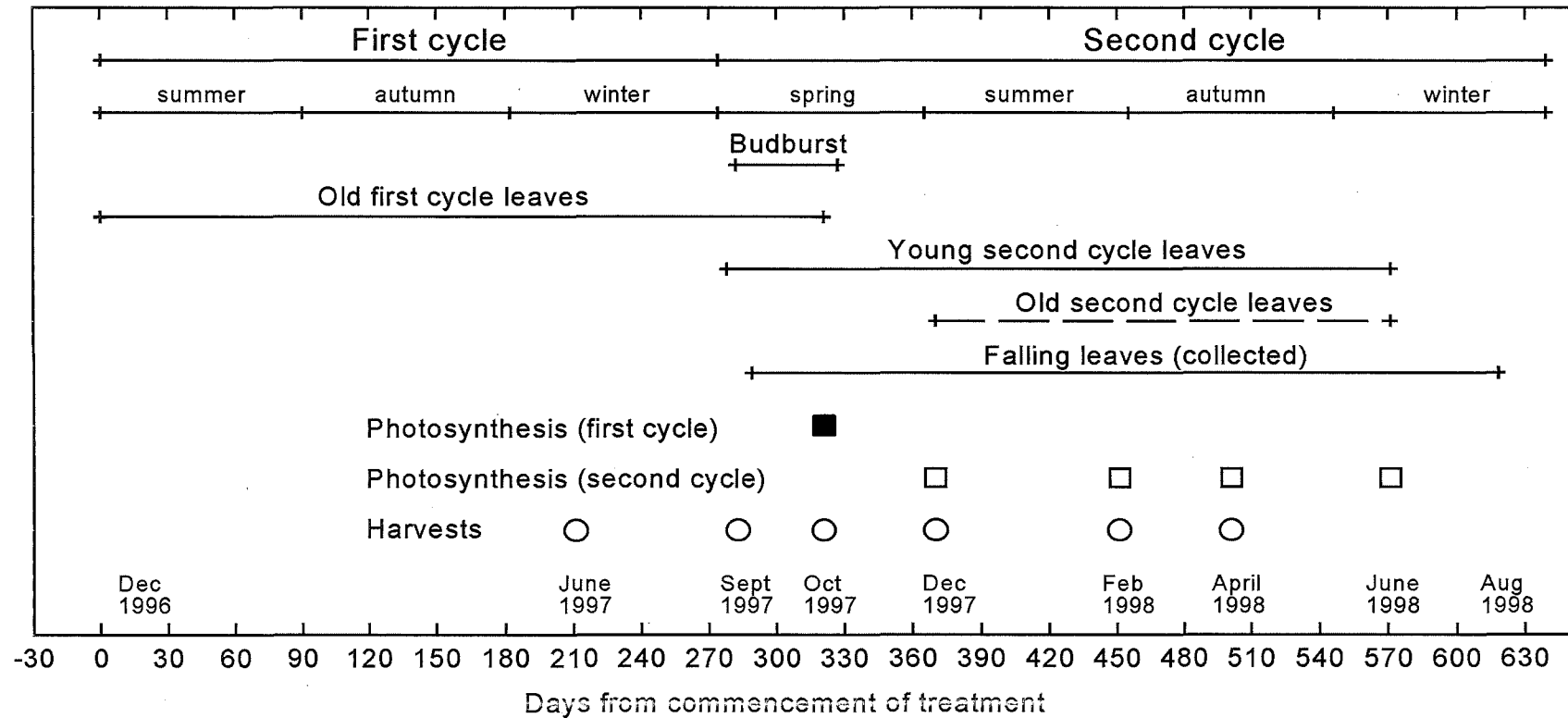


Figure 2.1. Diagram showing timing and duration of budburst and leaf growth and loss throughout the experimental period. Also shown are the dates for harvests and measurements of photosynthesis.

Three broad classes of leaves existed in the canopy (Fig. 2.1). “Old first cycle leaves” were extant leaves from the first cycle and still remaining on the trees in June, September and October. Some of these persisted into the second cycle, but numbers and longevities are not known, and it was assumed that they all fell by late summer. Leaves growing during the second cycle fell into two classes. The first class, described as “young second cycle leaves” were visibly healthy. The second class, described as “old second cycle leaves”, were discoloured and in the course of abscission. Leaves falling between days 289 and 618 were collected periodically during the experiment. Subsamples from bulk canopy young and old leaf samples from the three second cycle harvests in summer and autumn, were analysed for nitrogen concentration using an ANA-SIRA mass spectrometer (V G Isogas, Middlewich, Cheshire, UK). Subsamples from the same bulk samples were also analysed for carbon isotopic composition ($\delta^{13}\text{C}$) using a TracerMAT continuous flow -- isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany), using an organic rich soil as the reference material, and wheat flower as a quality control, and standardised using IAEA standard NBS22.

2.2.3 Measurements of photosynthesis

Measurements of photosynthesis were made on one leaf of each tree prior to harvest. The trees were well watered in the evening prior to measurement. Measurements of photosynthesis, A , were made on single sun leaves (in the same position) from midway up the canopy, using a portable photosynthesis system (model LI-6400 LiCor Inc., USA) and an artificial light source (model 6200-02B). Leaves were equilibrated 15 minutes at saturating CO_2 concentrations and irradiance, Q , of $1500 \mu\text{mol mol}^{-1}$ and $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ respectively, and a

constant cuvette temperature of 20°C. Measurement of A were made as the CO_2 concentration in the chamber was automatically reduced in 12 steps down to 20 $\mu\text{mol mol}^{-1}$, to determine the A/c_i (where c_i is the intercellular CO_2 concentration) curve. A similar procedure was used for the A/Q curve. The CO_2 concentration in the cuvette was returned to 350 $\mu\text{mol mol}^{-1}$, and with Q being reduced in 12 steps down to darkness. $A_{\text{max}Q}$ (maximum rate of photosynthesis at ambient CO_2 concentration and saturating irradiance) and quantum efficiency, α , were determined by fitting the A/Q data to a non-rectangular hyperbola (Farquhar & Wong 1984). V_{cmax} (maximum rate of carboxylation activity by rubisco when substrates RuBP and CO_2 are saturating) and J_{max} (maximum rate of electron transport at saturating irradiance) were determined by following the coupled photosynthesis-stomatal conductance model for individual leaves described by Leuning (1995), and fitting the A/c_i data to the model for photosynthesis of Farquhar et al (1980) using non-linear least squares procedure. Equations are shown in the Appendix to this thesis. Leaf area was determined using photosensitive paper.

2.2.4 Statistical analysis

Two way analysis of variance (ANOVA) was used to test for the main and interactive effects of time and treatment. Differences were considered significant if probabilities (P) were less than 0.05. Where ANOVA assumptions were met marginally, probabilities less than 0.01 were required. In all cases, log transformation was necessary before undertaking the ANOVA and means and standard errors are reported after retransformation, with assymetric error bars calculated from mean standard errors (MSE) (Maindonald & Cox, 1984; Fowler & Cohen 1992). Comparisons by t test were made assuming unequal variances.

2.3 RESULTS

2.3.1 Timing and duration of budburst

There were no significant differences between treatments in the date of commencement of budburst. Budburst occurred first in MN trees (day 286), next in LN trees (day 289), and lastly in HN trees (day 291). There was also no significant difference between treatments, in the interval between the first budburst within a treatment, and the last budburst in that treatment. These were 38, 29 and 36 days respectively. The mean duration of budburst on individual trees was $17 (\pm 1)$ days in HN and MN trees, but was significantly lower in LN trees, $12 (\pm 1)$, ($P < 0.01$).

2.3.2 Bud numbers

Nitrogen supply during the first cycle affected the total number and mass of overwintering buds present on each tree at the start of the second cycle (Fig. 2.2a,b). HN trees carried 1.5 times (by number) and 2.3 times (by mass) more buds than MN trees, which in turn carried almost 4 times more (in both cases) than LN trees ($P < 0.001$ in all cases).

2.3.3 Height, basal area and branch numbers

With the exception of LN trees (which increased in height until winter, $P < 0.001$) there was little change in height or basal area for all trees during the period between mid-autumn 1997 (day 131) and budburst in spring (Figs. 2.3a,b). Branch numbers did not change between June and October harvests in 1997 (Fig. 2.3c). Following budburst, all trees commenced growing in height, basal area and branch numbers and, (with the exception of HN basal area and LN branch numbers both of which peaked by late summer), this continued at least until mid-autumn. During the

measurement period, mean tree height increased from 343 mm to 724 mm (± 301 mm; 95% confidence interval), from 248 mm to 753 mm (± 321 mm), and from 138 mm to 260 mm (± 104 mm) for the HN, MN and LN treatments respectively. Concomitant changes in basal area were from 11 mm² to 107 mm² (± 70.1 mm²), from 6 mm² to 113 mm² (± 48.1 mm²), and from 3 mm² to 16 mm² (± 8 mm²). The

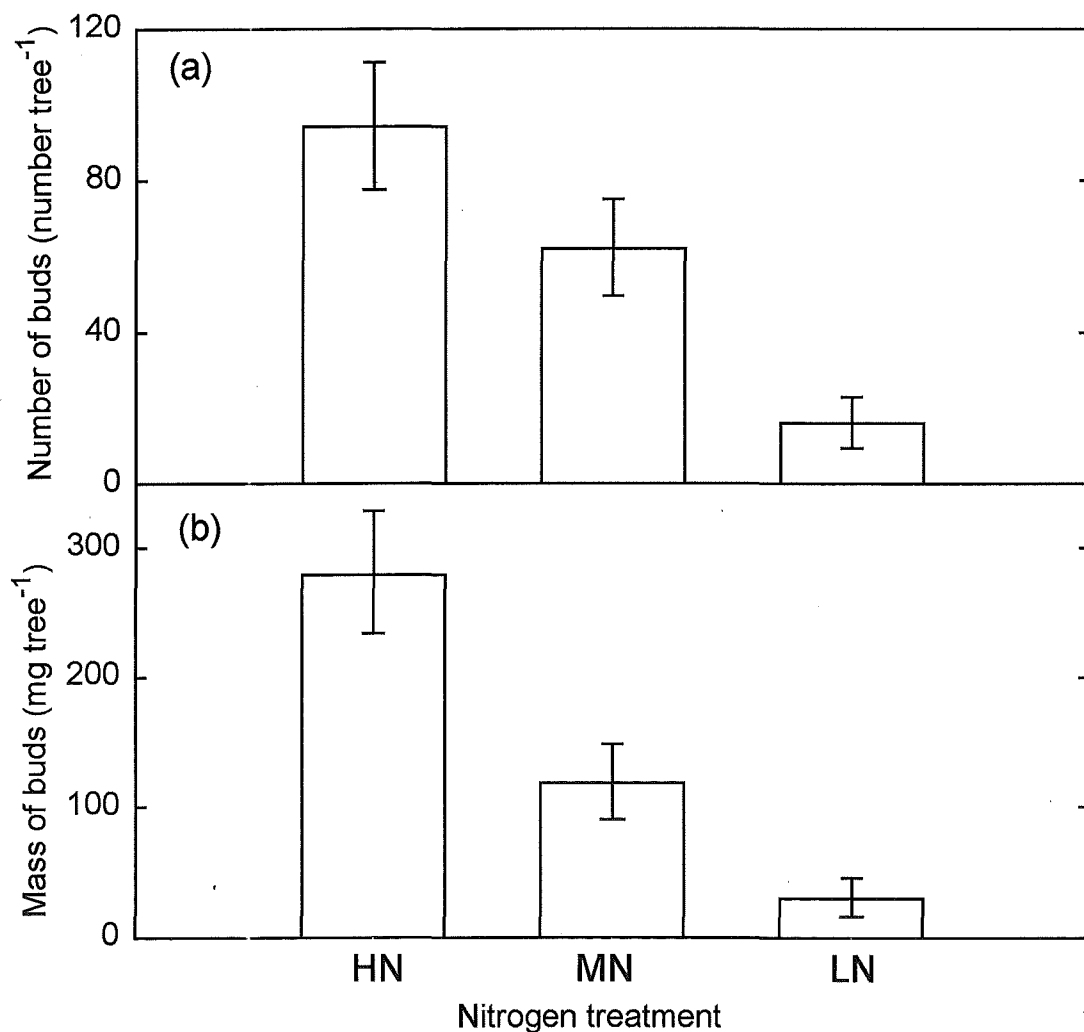


Figure 2.2. Mean overwintering bud numbers (a) and mass (b) at day 211 for *Nothofagus fusca* trees grown at three different concentrations of nitrogen supply (HN = high, MN = medium, LN = low). Error bars are \pm SE.

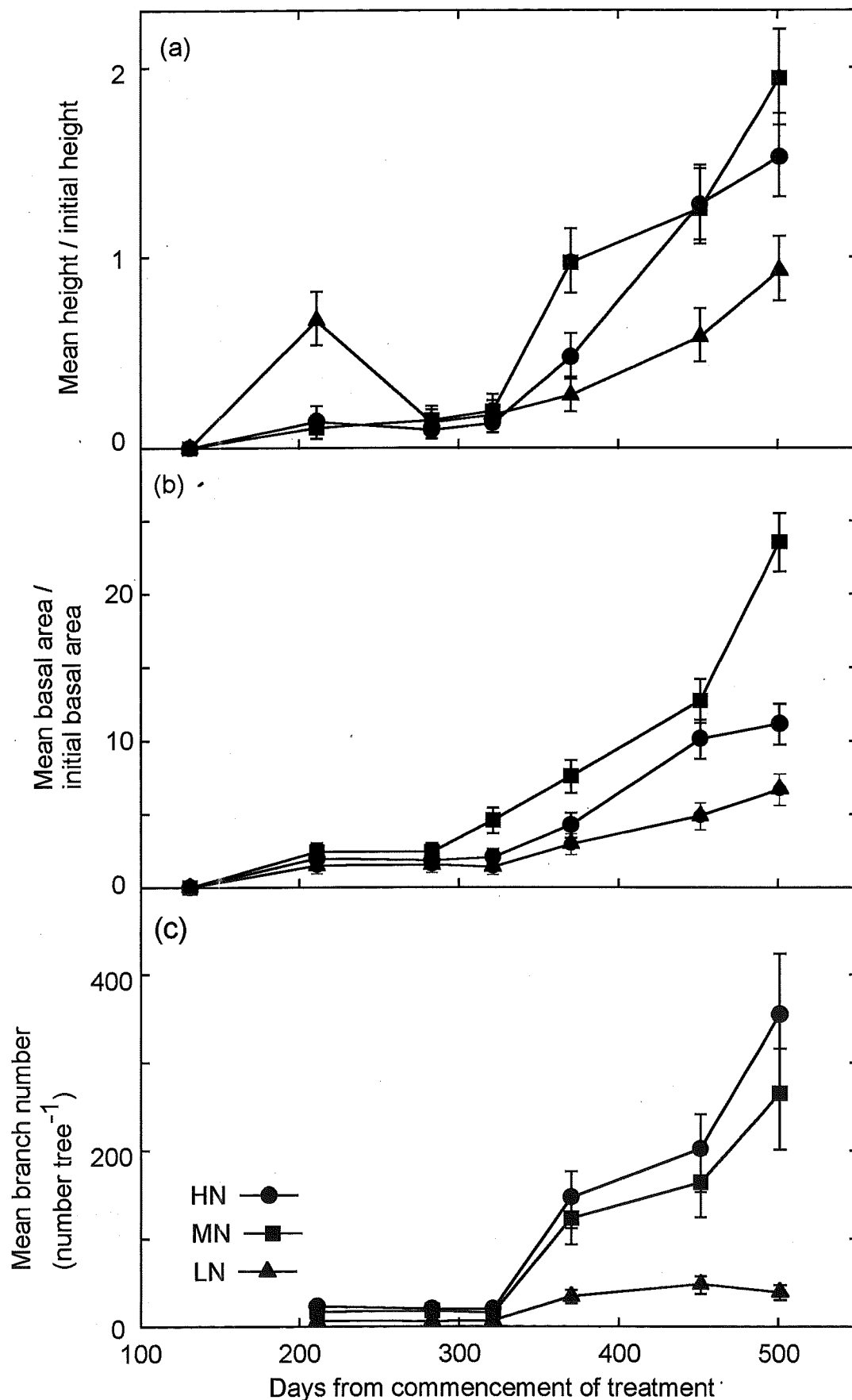


Figure 2.3. Changes in mean (a) height/initial height (b) stem basal area/initial basal area, and (c) stem branch numbers of *Nothofagus fusca* trees grown at three different concentrations of nitrogen supply (HN = high, MN = medium, LN = low). Error bars are \pm SE.

Relatively greater height and basal area growth rates in HN and MN trees compared with LN trees, was not significant until after the December harvest ($P \leq 0.006$). However, the immediate increase in branch numbers for both HN and MN trees, when compared with LN trees at the same times during the same period, was dramatic and significant ($P < 0.001$). HN and MN trees had twice as many branches as LN trees at budburst, but this rose to four times the number in December and February, and eight times the number by April (Fig. 2.3c). The increase in LN tree branch numbers ceased before the end of summer, while branch numbers continued to increase for HN and MN trees up to mid-autumn. While the trend was for HN trees to grow more branches, height growth lagged behind that for MN trees after summer (Fig. 2.3a), and basal area increment virtually stopped, (the difference with MN trees becoming significant by day 501; $P \leq 0.006$; Fig. 2.3b). By comparison, MN trees continued to increase in height from budburst onwards, and basal area even increased during autumn.

2.3.4 Increase in leaf numbers, area and mass

During winter of the first cycle, leaf numbers remained reasonably constant (Fig. 2.4a). Following budburst in the second cycle there was a rapid increase in total leaf numbers for all trees and this continued to autumn. In HN trees, total leaf numbers at budburst increased 6 times by the beginning of summer (day 370), 16 times at the end of summer (day 451), and 22 times by mid-autumn (day 501). Increases in MN trees, (not significantly different), were 6 times, 13 times and 19 times. Increases were much smaller in LN trees than for the other treatments ($P < 0.001$), being 3 times at the beginning of summer (day 370), and levelling off at 7 times by the end of summer (day 451). The number of old leaves still held on the tree at

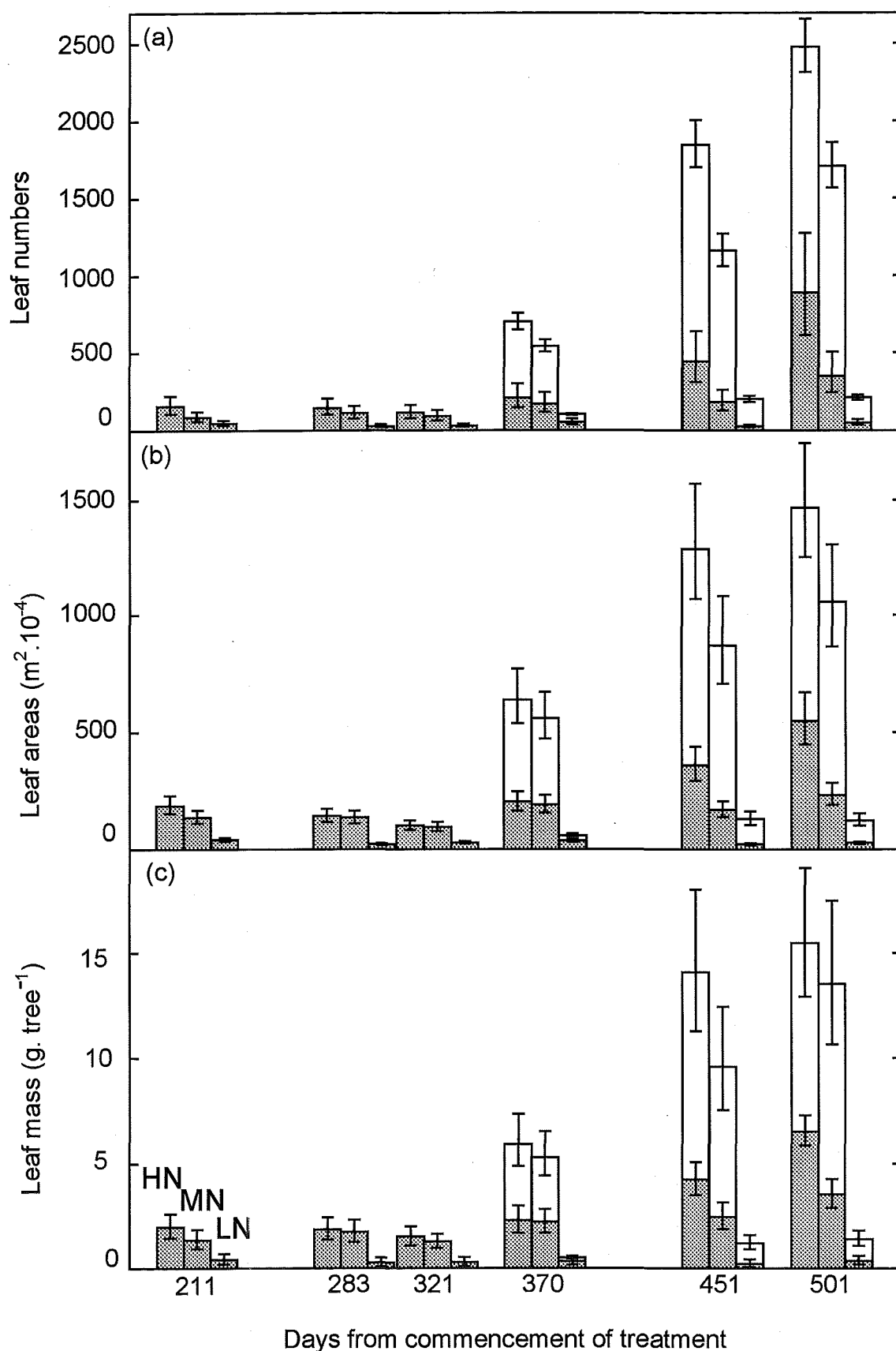


Figure 2.4. Changes in mean values for old (filled bars) and young (open bars) (a) leaf number, (b) leaf area, and (c) leaf mass for *Nothofagus fusca* trees grown at three different concentrations of nitrogen supply (HN = high, MN = medium, LN = low). Error bars are \pm SE.

harvest increased in HN and MN trees during the period from budburst to the following autumn (day 451). This effect was most marked in the HN trees where the number of old leaves had almost doubled at the beginning of summer, was almost 4 times higher at the end of summer, and had risen almost 8 times by mid-autumn. These represented 30%, 25% and 36% of total leaves then in the canopy. The difference with MN trees, where old leaf numbers increased by 2 times (31%), 2 times (16%) and 4 times (20%) was not significant. In contrast, numbers of old leaves in LN trees showed little change, and this difference with HN and MN trees was significant ($P < 0.01$). Old leaves initially represented a high proportion of total leaves, (50%), then less, (13%) and finally, 23% of total. The same trends were evident in comparisons of seasonal changes in leaf area and mass of total canopy and old leaves (Fig. 2.4b,c). However, the proportional changes in total canopy leaf mass and total canopy leaf area, between the beginning (day 211) and end (day 501) of measurement were lower than for changes in leaf numbers. Total leaf numbers in HN trees increased approximately 16 times during that period, but total leaf areas and total leaf mass both increased 8 times. The proportional changes for MN trees during the same period were respectively, 20 times, 8 times, and 10 times, and for LN trees were 5 times, 3 times, and 4 times.

2.3.5 Leaf fall

In all trees, leaf fall remained fairly constant from budburst to the end of spring (day 366) (Fig. 2.5a). There was little change in cumulative totals from then until late summer (day 424) when leaf fall in the HN trees increased suddenly, continuing at an approximately linear rate right through winter to early August (day 618), three weeks before spring (Fig 2.5b). There was an initial steady increase in leaf loss

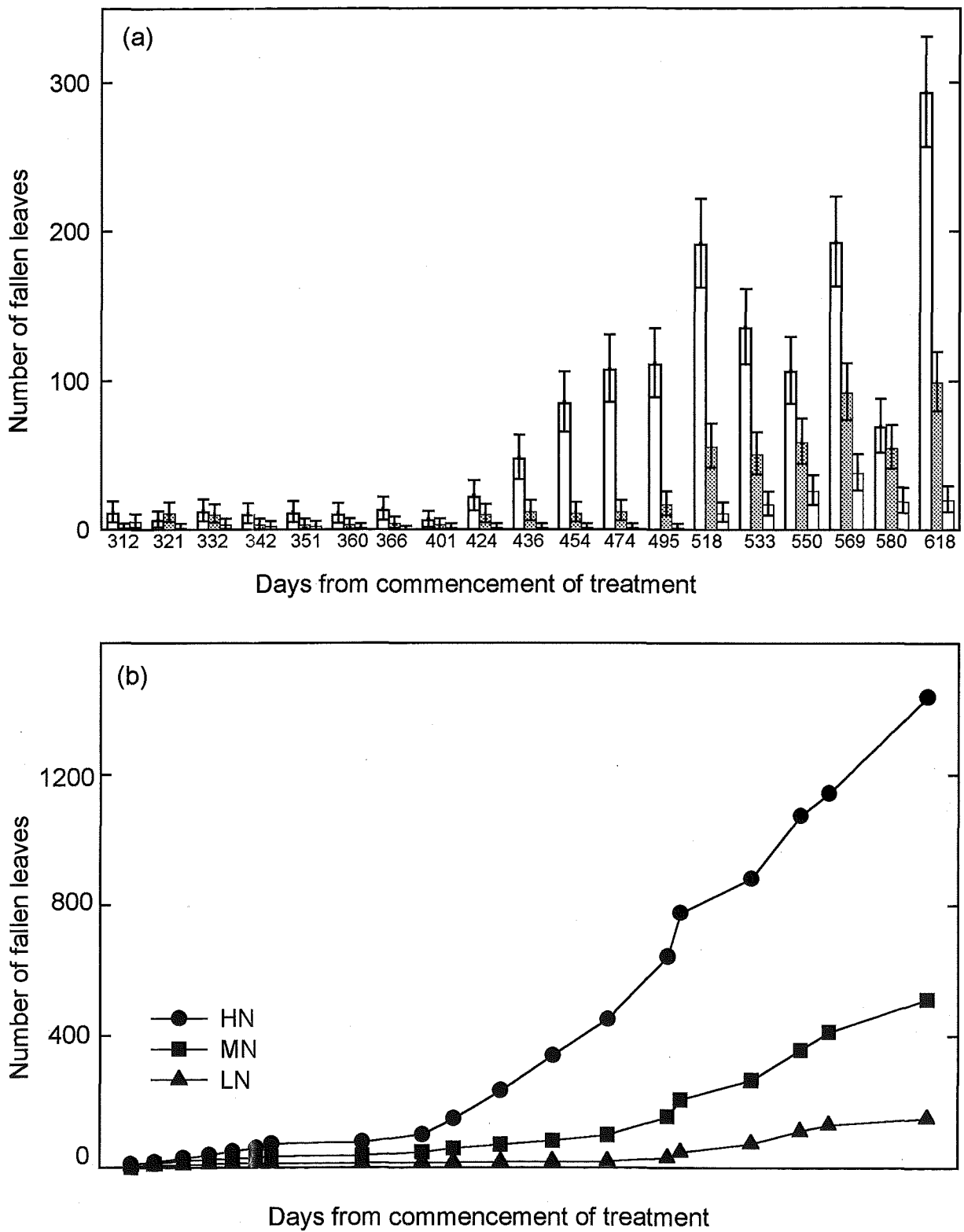


Figure 2.5. (a) Mean (high HN left, medium MN centre, low LN right) and (b) cumulative numbers of fallen leaves collected between days 289 and 618 from the *Nothofagus fusca* trees. Error bars are \pm SE.

from MN trees from late summer (day 424) to mid-autumn (day 495), followed by a sudden increase in the rate which remained constant until early August (day 618), (Fig 2.5b). Few leaves were lost from the LN trees over the autumn period (to day 495), after which leaf loss commenced and continued at approximately the same rate until mid-winter (day 618) (Fig 2.5b). Repeated measures anova showed these different time*treatment interactions were all highly significant ($P < 0.001$).

Differences in leaf loss in relation to total leaf number are summarised in Table 2.2. The greatest proportional losses of leaves falling from the existing canopy did so in two pulses, irrespective of the nitrogen treatment, the first during spring and the second between mid-autumn and late winter (late summer and late winter in HN trees). During spring (between days 289 and 366), falling leaves represented approximately half of all the old leaves on HN and LN trees and about a third of those on MN trees at the end of winter. Yet, at the end of spring (day 367) the numbers of old leaves (second cycle) on trees had increased to levels representing almost a third of all leaves on HN and MN trees, and half those held on LN trees. Accordingly, a large number of leaves generated during this early part of the second cycle were already moribund at the start of summer. During the second major flush (mid-autumn to late winter, days 496 to 618), almost 67% of the total canopy fell from LN trees, slightly less than 50% from HN trees and 25% from MN trees. Only during this second pulse of leaf loss, did populations of falling leaves exceed numbers of old leaves in the canopy at the start of the period. Falling leaves were not collected during the last phase of the first cycle, but comparison of leaf numbers for June (day 211) and September 1997 (day 283) (Fig. 2.4a), suggested a similar response. The reduction in leaf numbers between those times represented 8%

of total leaves on HN trees in June and more than 30% on LN trees, but there were no losses from MN trees.

During 10 weeks between late summer and mid-autumn (days 424 to 495), preceding the second pulse of leaf loss, all trees continued to expand their canopy areas through the addition of new leaves. Notwithstanding this, a large number of leaves began falling from HN trees during that time, representing 20% of the extant canopy. While this appeared to mark an earlier commencement of the second pulse in HN trees (Fig. 2.5a,b) it can be distinguished from commencement of the second pulse in MN and LN trees which was either shortly before or after growth quiescence.

During the periods shown in Table 2.2 precipitation (mm), and maximum and minimum air temperatures (°C) were respectively 80.5, 18, 17.2 (spring), 141.5, 19.7, 10.6 (summer), 81.6, 19.7, 11.5 (late-summer to mid-autumn), and 226.1, 12.9, 3.1 (mid-autumn to late winter). Of the 81.6 mm rainfall during late summer to mid-autumn, 30 mm fell in one day (day 461), and so trees experienced severe drought during that period.

2.3.6 Leaf nitrogen concentration and specific leaf area

Nitrogen concentrations on a mass basis (N_m) for old leaves, showed little change over winter and spring until summer (day 370), when they decreased, reaching lowest levels ($P < 0.001$) by mid-autumn (day 501) (Fig. 2.6a). At days 211 (first cycle winter) and 321 (budburst), N_m values for old leaves of LN trees were significantly lower than those for HN and MN trees ($P < 0.001$). During the following summer and autumn (days 370 to 501), old leaf values of N_m for both LN and MN trees were significantly lower than for HN trees ($P \leq 0.011$).

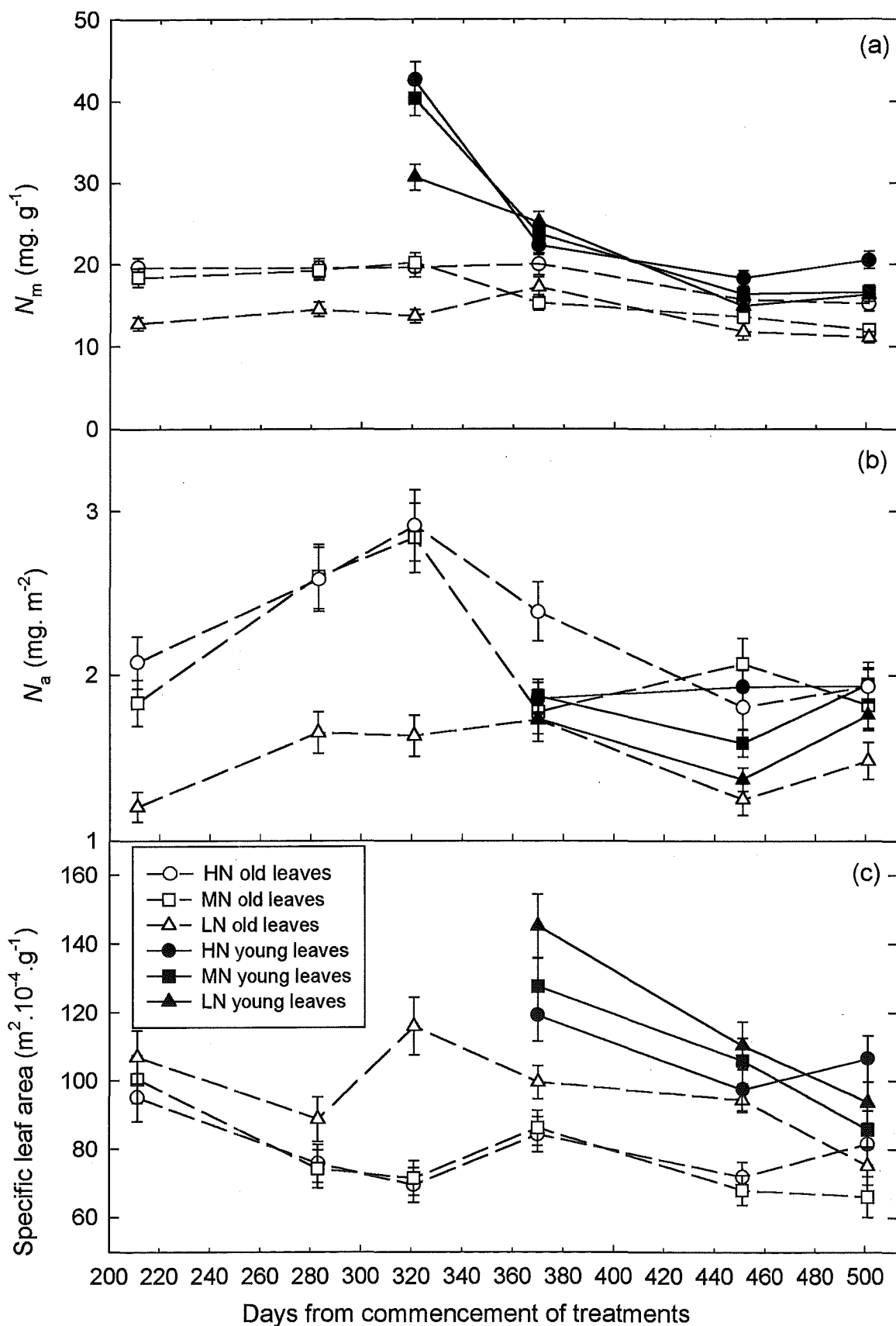


Figure 2.6. Changes in mean leaf nitrogen concentration on (a) mass basis, N_m , and (b) area basis, N_a , for old and young leaves of *Nothofagus fusca* trees grown at three different concentrations of nitrogen supply (HN = high, MN = medium, LN = low), and (c) corresponding specific leaf areas. Error bars are \pm SE.

N_m values for young leaves were greatest when they were emerging and expanding between budburst and early summer ($P < 0.001$). By late-summer and autumn, N_m values approached those for old leaves at the start of the preceding winter (day 211), and spring (days 283 and 321) (Fig. 2.6a). Reductions in N_m for young leaves from budburst to late summer were significant ($P < 0.001$), but changes from then on were not. N_m for young leaves emerging at budburst were significantly greater for HN trees and significantly lower for LN trees. ($P \leq 0.004$). However there were no differences between treatments over the summer period until mid-autumn, when values in HN trees were significantly higher than those in MN and LN trees ($P \leq 0.004$). Regardless of nitrogen supply, N_m values for young leaves were always higher than those of contemporaneously old leaves during summer and autumn of the second cycle ($P < 0.05$).

Nitrogen concentrations on an area basis (N_a), for old leaves of all trees increased during winter of the first cycle, before decreasing over summer to levels approximating those at the start of the preceding winter (Fig. 2.6b). In HN and MN trees the increase over winter was significantly greater than for LN trees ($P < 0.001$), and continued at least until budburst. In LN trees, N_a levelled off before spring. Values of N_a for LN trees were always significantly lower than those for HN and MN trees during winter ($P < 0.001$). There were, however, no significant differences in N_a between young and old leaves within any treatment during summer and autumn.

Specific leaf area, S , of both old and new leaves respectively, did not differ between treatments, except that values for old LN leaves were higher at budburst (day 321), and at the end of summer (day 451) ($P < 0.001$). Nor were there any significant differences between S for first cycle old leaves (day 211), and those for

second cycle new leaves at the end of summer (day 451), and at mid-autumn (day 501) (Fig. 2.6c). Apart from LN trees in late summer, S for new leaves of all trees were always significantly higher than those of contemporaneous old leaves ($P < 0.05$).

2.3.7 Photosynthesis measurements

Values of $A_{\max Q}$ were low at budburst, ($4\text{--}7 \mu\text{mol m}^{-2} \text{s}^{-1}$ for old first cycle leaves), and in winter ($3\text{--}5 \mu\text{mol m}^{-2} \text{s}^{-1}$ for young second cycle leaves), increasing during summer ($9\text{--}12 \mu\text{mol m}^{-2} \text{s}^{-1}$ for young second cycle leaves), then decreasing through autumn (Fig. 2.7a). There was some indication of a similar trend in quantum efficiency, α , but the seasonal changes were not significantly different (Fig. 2.7b). Values for V_{\max} ($P < 0.05$) and J_{\max} ($P < 0.001$) (Fig. 2.7c,d) were significantly lower at budburst than at other times during the year. The mean ($\pm 95\%$ confidence interval) ratio of J_{\max} to V_{\max} was 2.1 ± 0.17 .

2.3.8 Stable carbon isotopic composition of young and old leaf populations

Mean $\delta^{13}\text{C}$ values for old and young leaves in December, February and April were higher for the HN treatment and lower for the LN treatment (Table 2.1, $P < 0.001$). However there was no significant difference in $\delta^{13}\text{C}$ values between old and young leaves regardless of nitrogen supply, and no seasonal change in those values during summer-autumn conditions.

2.4 DISCUSSION

Young *Nothofagus fusca* trees responded to increased nitrogen supply by increasing total photosynthetic area, without any changes to the photosynthetic capacity of

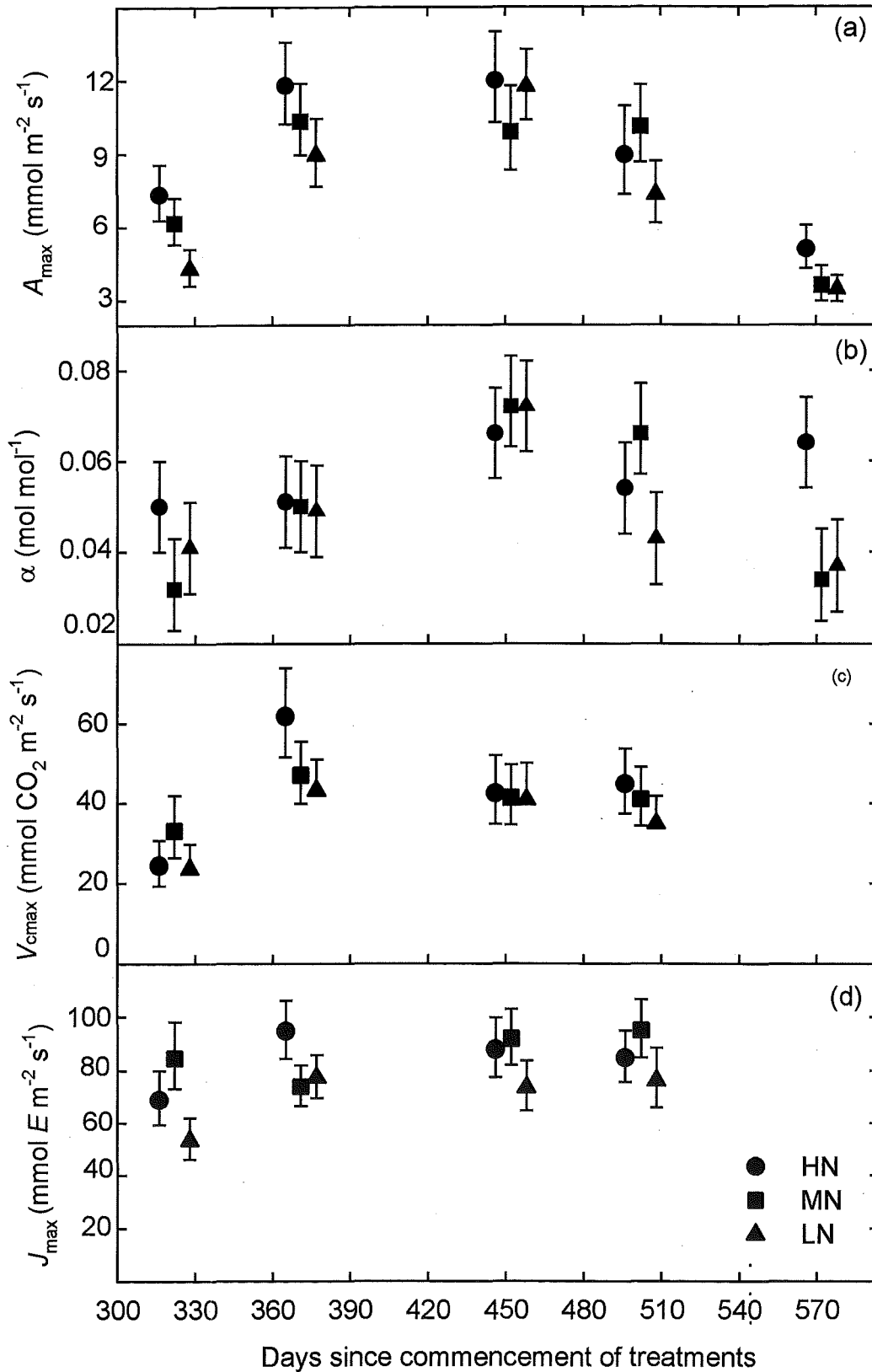


Figure 2.7. Changes in maximum rate of, $A_{\max Q}$, quantum efficiency, α , maximum rate of carboxylation activity by rubisco when substrates RuBP and CO₂ are saturating, V_{\max} and maximum rate of electron transport at saturating irradiance, J_{\max} for leaves of *Nothofagus fusca* trees grown at three different concentrations of nitrogen supply (HN = high, MN = medium, LN = low). Error bars are \pm SE.

Table 2.1 Mean $\delta^{13}\text{C}$ values of old and young (second cycle) leaves in December 1997, and February and April 1998, for *Nothofagus fusca* trees grown at three different concentrations of soil nitrogen supply (HN = high, MN = medium, LN = low).

| N treatment | Leaf age | December 1997 | February 1998 | April 1998 |
|-------------|----------|----------------|----------------|----------------|
| LN | old | -28.3 (0.11) a | -28.1 (0.09) a | -28.4 (0.09) a |
| | young | -28.0 (0.09) a | -28.3 (0.09) a | -28.1 (0.09) a |
| MN | old | -27.0 (0.09) b | -26.5 (0.09) b | -26.7 (0.09) b |
| | young | -26.5 (0.08) b | -26.6 (0.09) b | -26.8 (0.09) b |
| HN | old | -26.3 (0.19) c | -26.0 (0.19) c | -26.4 (0.09) c |
| | young | -26.3 (0.16) c | -26.0 (0.16) c | -26.4 (0.09) c |

Figures in brackets are \pm SEs. Significant differences between treatments are indicated by different letters ($P < 0.05$).

Table 2.2 Mean total leaves falling during spring, summer, late-summer to mid-autumn, and mid-autumn to late winter, from *Nothofagus fusca* trees grown at three different concentrations of soil nitrogen supply (HN = high, MN = medium, LN = low). as percentages of mean old, and mean whole canopy, leaf totals at the start of each period.

| Period | Treatment | Total old leaves at start of period | Leaves falling during period | Falling leaves as % of old leaves | Total leaves in canopy at start | Falling leaves as % of canopy total |
|---|-----------|--|---------------------------------|--------------------------------------|------------------------------------|--|
| spring (days 289 to 366) | HN | 142 | 73 | 51 | 142 | 51 |
| | MN | 109 | 35 | 32 | 109 | 32 |
| | LN | 27 | 14 | 47 | 27 | 52 |
| summer (days 367 to 423) | HN | 209 | 6 | 3 | 700 | < 1 |
| | MN | 169 | 3 | 2 | 542 | < 1 |
| | LN | 51 | 1 | 2 | 102 | 1 |
| late-summer to mid-autumn (days 424 to 495) | HN | 444 | 373 | 85 | 1847 | 20 |
| | MN | 182 | 62 | 34 | 1160 | 5 |
| | LN | 26 | 5 | 20 | 201 | 2.5 |
| mid-autumn to late winter (days 496 to 618) | HN | 886 | 986 | 111 | 2478 | 40 |
| | MN | 350 | 412 | 118 | 1709 | 24 |
| | LN | 49 | 131 | 267 | 211 | 62 |

Table 2.3 Mean leaf characteristics of leaves in June 1997 and April 1998, for *Nothofagus fusca* trees grown at three different concentrations of soil nitrogen supply (HN = high, MN = medium, LN = low).

| Leaf parameters | June 1997 | | | April 1998 | | |
|--|--------------------------|--------|--------|---------------------------|--------|--------|
| | All leaves (first cycle) | | | All leaves (second cycle) | | |
| | LN | MN | HN | LN | MN | HN |
| Leaf number (tree ⁻¹) | 44 a | 84 b | 155 b | 211 c | 1709 d | 2478 d |
| Leaf area (m ² .10 ⁻⁴ tree ⁻¹) | 41 a | 136 b | 187 b | 124 c | 1057 d | 1467 d |
| Leaf dry weight (g tree ⁻¹) | 0.4 a | 1.3 b | 2.0 b | 1.4 c | 13.5 d | 15.5 d |
| Mean area per leaf (m ² .10 ⁻⁴ leaf ⁻¹) | 0.9 a | 1.6 b | 1.2 b | 0.6 c | 0.6 c | 0.6 c |
| Specific leaf area (m ² . 10 ⁻⁴ g ⁻¹) | 104 a | 101 a | 95 a | 89 a | 78 a | 95 a |
| N content per leaf (mg N leaf ⁻¹) | 0.11 a | 0.30 b | 0.24 b | 0.10 a | 0.12 a | 0.11 a |
| N content per unit mass (mg N g ⁻¹) | 13 a | 18 b | 20 b | 16 b | 17 b | 19 b |
| N content per unit area (mg N m ⁻² . 10 ⁻⁴) | 1.2 a | 1.8 b | 2.0 b | 1.6 b | 1.9 b | 1.9 b |
| N content per tree (mg N tree ⁻¹) | 5 a | 25 b | 39 b | 21 c | 205 d | 273 d |

Significant differences between treatments are indicated by different letters (P < 0.05).

individual leaves. However, there were important developmental differences between the first and second cycles, illustrated in the summary of data in Table 2.3. During the first cycle (day 211), the increased photosynthetic surface area was due to increases in both average leaf area (MN) and the number of leaves per tree (especially HN). Also, mean values of area and nitrogen concentration and content of individual leaves were lower in LN trees. In contrast, during the second cycle (day 501), the dominating response was an increase in the total number of leaves, and values for area and nitrogen concentrations and content of individual leaves were the same for all treatments. Also, total leaf area, weight and number increments for MN and HN trees were between two and five times greater, and whole tree nitrogen content increments were respectively doubled, and increased by half, during the second cycle.

Mean foliar nitrogen content (as a percentage of dry weight) for all treatments ranged between 1 and 3.02 mg. g⁻¹ (data not shown), and was generally higher than the 1.6 to 1.8 mg. g⁻¹ (Heine, 1973) and 1.3 to 1.72 mg. g⁻¹ (Adams, 1976) reported for mature forest trees. No differences in $A_{\max Q}$, α , V_{\max} or J_{\max} were demonstrated between nitrogen treatments (Fig. 2.7) showing that the dominant effect of an increased nitrogen supply was to increase total leaf area rather than to induce an increased rate of photosynthesis. Values for $A_{\max Q}$ were typical of broad-leaved deciduous hardwoods (3.8 to 14.8 $\mu\text{mol m}^{-2} \text{s}^{-1}$) reported by Reich *et al.* (1995). In *N. fusca* trees, $A_{\max Q}$ reached maximum values when measured seven weeks after budburst, although there was a trend suggesting that this might occur later in LN trees (Fig. 2.7). Assimilation during favourable periods in winter, can offset costs of maintaining evergreen foliage, but photosynthetic capacity may be reduced. At the start of first cycle winter (day 211), $A_{\max Q}$ was half the peak summer values for the

second cycle. Variation in $A_{\max Q}$ is largely due to differences in rubisco activity (Field & Mooney, 1986; Reich *et al.*, 1998). Consistently with this, seasonal changes in V_{\max} followed those for $A_{\max Q}$ (Fig. 2.7c). Values were typical of temperate forest hardwoods ($47 \pm 33 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) as reported by Wullschlegger (1993). Values for α (Fig. 2.7b) were about the approximate value of $0.053 \text{ mol CO}_2 \text{ mol}^{-1}$, given by Leegood (1993). The mean ratio of J_{\max}/V_{\max} was consistent with the general value of 2.68, (subject to variation between climatic zones, and in the relationship itself, for particular species), reported by Leuning (1997) after adjustment of reported results to a common temperature of 20°C .

During both cycles all values for S (Fig. 2.6c) were intermediate between those for trees with long-lived leaves and those for trees which are fast growing, as reported for North American temperate tree species by Reich *et al.* (1998). Values for N_m and N_a were also consistent with those for deciduous broadleaf trees from that biome (Reich *et al.*, 1995).

However, regardless of treatment, mean seasonal values of N_m , N_a and S for the whole canopy (Fig. 2.6) did not vary with the seasonal changes in $A_{\max Q}$ values for leaves from the outer edge of the canopy. Instead, values of S , N_a and N_m for old first cycle leaves at the start of winter, when $A_{\max Q}$ was lowest (day 211), were similar to those for mid-autumn young second cycle leaves (day 501), when $A_{\max Q}$ was maximum. Also, values of S , N_a and N_m for old second cycle leaves declined after budburst, reaching minimum values by mid-autumn (day 501). This class of leaves was moribund and destined to fall entirely in the last phase of the second cycle. Taken together, these observations indicated that whole canopy N_a , N_m and S , at days 211 (first cycle winter), 321 (start of second cycle summer) and 501 (mid-autumn of the second cycle) were generally the same. This was with the exception,

in all cases, for the old leaf fraction destined to fall in the last phase of the second cycle.

Studies of responses of other hardwood trees to an enhanced nitrogen supply have produced varying results. Increased total leaf area unaccompanied by change in photosynthetic capacity of individual leaves, seen in *N. fusca*, has also been observed in *Eucalyptus globulus* (Pereira *et al.* 1992). The response of Balsam Spire (a *Populus* cultivar hybrid tree) was also similar. Reduced nitrogen supply resulted in reduced total leaf area, without any change to photosynthetic capacity (Ibrahim *et al.*, 1997). However it contrasted with *Acer saccharum* seedlings which, under low-light subcanopy conditions, responded with increases in photosynthetic capacity alone (Walters & Reich 1997). There was also a contrast with *Ulmus americana* seedlings, which showed increases in both total leaf area and photosynthetic capacity (Walters & Reish, 1989). Increases in total leaf area resulting from increases in both the number and area of individual leaves, seen in *N. fusca* in the first cycle, was reported for both *Ulmus americana* (Walters & Reish, 1989), and Balsam Spire (Ibrahim *et al.*, 1997). Enlarged total leaf area resulting from increased area of individual leaves (but not their number) has been reported for both *Populus trichocarpa* x *Populus deltoides* hybrid trees (Heilman & Xie, 1994), and *Liquidambar styraciflua* seedlings (Kuers & Steinbeck, 1998). An increase in leaf numbers alone, seen in the second cycle (Table 2.2), was reported for *Populus tremuloides* (Coyne & Van Cleve, 1977). These studies by Coyne & Van Cleve (1977), Heilman & Xie (1994) and Kuers & Steinbeck (1998) did not report any investigation of accompanying changes in photosynthetic capacity. It has been suggested that species in which leaf number per shoot is determinate show large variations in leaf size, whereas species with a variable leaf number generally

respond to improved nutrient status primarily through a change in leaf number (Chapin, 1980). Leaf production in temperate woody trees may be completely pre-formed in the winter resting bud, or it may be complemented by neoformed leaves produced during the season of shoot expansion, and this can vary between species or even cultivars. For example comparison of four clones of *Fraxinus pennsylvanica* showed variation in the amount of leaf preformation, and this was considered to be due to environmental influences on particular genotypes (Remphrey & Davidson, 1994). These processes appear not to have been examined in *N. fusca*. However, overwintering buds of the related *N. dombeyi* produced two classes of shoots, one bearing apices that persist through the growing season, and the other, not. In both classes, there is an extension peak in spring at which time leaf numbers coincide with leaf primordia pre-formed in buds during the preceding season. Shoots with persisting apices produced axillary (or sylleptic) branches, provided they exceed threshold extension rates in spring, and again in summer (Puntieri *et al.* 1998). In the present study, an initial adjustment of internal nitrogen status to the new rates of supply could be expected before trees reached a stable growth rate characteristic of the particular treatment (Ingestad & Lund, 1979). Imbalances between plant internal nitrogen status and nitrogen supply during a critical threshold period may well have been responsible for differences between treatments in leaf characteristics in the first cycle, not seen in the second cycle (Table 2.2). In particular, increased area of individual leaves of MN trees may have resulted from limitations on neoform production of axillary branches, and therefore leaf numbers, caused by lagging shoot extension rates in spring compared with HN trees.

In this study the greatest proportional losses of leaves falling from the existing canopy did so in two pulses, the first during spring, and the second between mid-

autumn and late winter (mid-summer and late autumn in HN trees) (Fig 2.5a.b; Table 2.2). The first pulse of leaf loss in spring was accompanied by contemporaneous leaf replacement. The second pulse during late autumn and winter was not. This may represent an intermediate strategy between full deciduousness (second pulse), and evergreenness (first pulse).

The early commencement of leaf fall in HN trees during the 10 weeks from late summer (day 424) to mid-autumn (day 495) while still experiencing new leaf growth, was related to nitrogen supply. Losses were much lower in MN (5%) and LN (2.5%) trees (Table 2.2). The process can be contrasted with leaf loss during the second pulse (from mid-autumn to late winter) when all trees were growth-dormant. It may have been due possibly to increasing evaporative demand resulting from summer water deficits. During the periods shown in Table 2.2 precipitation (mm), and maximum and minimum air temperatures (°C) were respectively 80.5, 18, 17.2 (spring), 141.5, 19.7, 10.6 (summer), 81.6, 19.7, 11.5 (late-summer to mid-autumn), and 226.1, 12.9, 3.1 (mid-autumn to late winter) (Hort Research, Lincoln). Of the 81.6 mm rainfall during late summer to mid-autumn, 30 mm fell in one day (day 461), and so trees experienced severe drought during that period. The process of drought “stress-avoidance” by loss of leaves has been reported for other species elsewhere (Escudero & del Arco, 1987; Pallardy & Rhoads, 1993; Wendler & Millard, 1996). While soils were always moist, the potted trees in this experiment were grown outdoors at an elevation and annual precipitation both well below the extremes recorded for this species in the field (Fig. 1.1). Water deficit could result from incapability of the hydraulic system to supply sufficient water for the leaf area being generated by new growth (Radin & Boyer, 1982). This may have reached a threshold in HN trees. Although measurement of stomatal conductances was not

available, higher $\delta^{13}\text{C}$ values were associated with the HN treatment, supporting an increase in water use efficiency with nitrogen supply (Table 2.1). There were no differences in photosynthetic capacity of individual leaves (Fig. 2.7), which suggests that increased water use efficiency was achieved through an overall reduction in transpiration rate. Additionally, drought stress avoidance through leaf loss may be expected to result in containment or reduction of overall canopy transpirational water loss. Consistently with this, there was no difference in $\delta^{13}\text{C}$ values between old and young leaf fractions of the canopy regardless of nitrogen supply and the absence of seasonal change in those values during summer-autumn conditions suggests that water use efficiency remained constant within any particular treatment. It can be assumed that old leaves were generated earlier in the same season when reduced humidity deficits would tend to result in lower $\delta^{13}\text{C}$ values and new leaves were generated subsequently when greater humidity deficits would tend to produce higher $\delta^{13}\text{C}$ values. If so, the absence of $\delta^{13}\text{C}$ differences within any treatment over time would support a hypothesis that leaves senesced before water stress inhibited photosynthesis anywhere in the canopy. This would also be consistent with observations made by Read & Farquhar (1991), after determining $\delta^{13}\text{C}$ values for 22 species of *Nothofagus* from South America, New Zealand, Australia, New Guinea and New Caledonia. Species from latitudes experiencing hot dry summers showed greatest discrimination, suggesting a physiological or morphological adaptation to allow photosynthesis to continue during dry periods, while avoiding water deficits (Read & Farquhar, 1991; Comstock & Ehleringer, 1992; Williams & Ehleringer, 1996). The earlier onset and accelerated rate of summertime leaf fall seen in HN trees (Fig. 2.5a,b) is consistent with this explanation. *Betula pendula* Roth. seedlings given increased nitrogen supply, but reduced water supply, also showed a similar

response, with an earlier onset of leaf senescence, and an earlier pulse in leaf fall (Wendler & Millard, 1996).

HN trees, despite trends for greater leaf and branch numbers, showed no increase in tree height and basal area, as might have been expected with the doubling of nitrogen supply from MN concentration (Fig. 2.3). A similar response to increased nitrogen supply was seen in *Liquidambar styracifolia* (Kuers & Steinbeck, 1998). A possible explanation (Brix & Ebell, 1969) is that, in the absence of an increased photosynthetic capacity, foliage growth might have been stimulated beyond an optimum in HN trees, at the expense of stem growth. Stem height and basal area growth might not have been sustainable due to the sink dominance of greater predetermined numbers of bud meristems, compared with cambial meristems in HN trees.

In conclusion, nitrogen allocation to an enlarged total leaf area may be more important than photosynthetic rate as a source of growth rate variations in moderately low irradiation (Walters *et al.*, 1993; Walters & Reich, 1996). The response seen in young *N. fusca* trees, even at high irradiance, is consistent with adaptation to suppression beneath an essentially evergreen overstorey. This suggests an important role for site nitrogen availability in competition between young trees in the understorey or upon release through a canopy gap, similar to *Acer saccharum* seedlings (Walters & Reich, 1997). *N. fusca* also tends to inhabit river terraces and the mid-slopes of inland valleys on deeper, more fertile soils (Ogden, Stewart & Allen, 1996), and foliar analysis suggests it has higher nutrient requirements (Adams, 1976). This implies some sensitivity to soil nutrient status. *N. fusca* seedlings are shade persistent, suppressed, and slow-growing beneath the closed canopy of parent trees, providing a relatively constant average advance growth

population from which regeneration proceeds (Kirkland, 1961; June & Ogden, 1975). Differences in the autumn to early-summer phenology of seedlings and mature trees may be important to seedling persistence and survival beneath parent trees, as seen in seedlings of *Acer saccharum*. and *Fagus grandifolia* (Gill *et al.* 1998).

2.5 SUMMARY

In contrast with winter-deciduous trees from the Northern Hemisphere, young *N. fusca* trees maintained an evergreen canopy on an annual basis. Certain phenological events remained unchanged by manipulations of N supply. First, the size of the photosynthesising area of the canopy was reduced by loss of leaves during the period from late autumn, until just before spring. Then a period of maximum loss of leaves occurred, about the time of budburst. This was accompanied by the emergence and expansion of new stems and leaves, so that trees were never devoid of leaves. Secondly, there was no change in the timing of onset of these processes. Thirdly, the principal method of adjustment to the C gathering mechanisms was, uniformly, by change to the numbers of leaves, i.e. a spatial adjustment.

In contrast with the retention of these strategies independently of changes in tree N-status, increased N supply resulted in increased leaf loss during the hot dry summer months of increased atmospheric drought. This appeared to be a process for avoiding drought-induced stress.

The implications of these characteristics for the uptake, storage and internal recycling of N, are considered in the next chapter. Their implications as a strategy

for synchronising seasonal growth, and C and N supply and demand for new growth, are discussed in Chapter 5.

CHAPTER 3

THE INFLUENCE OF NITROGEN SUPPLY ON GROWTH AND INTERNAL NITROGEN RECYCLING IN YOUNG TREES OF *NOTHOFAGUS FUSCA* (HOOK F.) OERST.

3.1 INTRODUCTION

The dynamics of internal seasonal recycling of nitrogen (N) have been studied and characterised in a number of broad-leaf tree species (Millard 1996), both deciduous (Weinbaum *et al.* 1987; Wendler & Millard 1996; Nielsen *et al.* 1997; Weinbaum & van Kessel 1998) and evergreen (Legaz *et al.* 1982; Legaz, Serna & Primo-Millo 1995; Wendler *et al.* 1995). These studies reported that both deciduous and evergreen species stored N over winter in perennial tissues, for remobilisation into new seasonal growth in spring. The site for storage in deciduous trees was in perennial woody tissues, including the bark of stems and, in some species, also the roots (Taylor & May 1967; Tromp 1983; Millard & Proe 1991; Millard 1996). In contrast, the site for N-storage in evergreen species was primarily in overwintering leaves, and not in roots (Feigenbaum *et al.* 1987; Legaz, Serna & Primo-Millo 1995; Wendler *et al.* 1995).

The process of winter storage and spring remobilisation has the effect of uncoupling peak growth demand for N from delays or shortages inherent in soil-N uptake during spring and early summer, because it is independent of soil-N availability at those times (Millard 1996). Quantitative estimates of the relative proportions of recycled N and N from soil/fertiliser pools in new growth have shown

that up to 70% of spring N demand (Legaz *et al.* 1995), and up to 60% of annual N demand (Weinbaum & van Kessel 1998), can be met from storage N.

Because of the contrast in N-storage sites of deciduous and evergreen species these mechanisms were examined in *Nothofagus fusca*, which is an evergreen tree that exhibits some deciduous seasonality in leaf turnover (Wardle 1984). It is also an important component of natural forest ecosystems in New Zealand. All species of *Nothofagus* endemic to New Zealand exhibit these characteristics to a greater or lesser extent (Wardle 1984). Deciduousness is most pronounced in *N. fusca* where mature trees commence shedding leaves at budburst and can appear relatively bare of foliage during the transition to new season's canopy (Russell 1936). This led to the hypothesis that leaves falling about the time of spring budburst would be the most likely site for N-storage. It also suggested that N would be remobilised directly from senescing leaves into new growth, in contrast to evergreen species such as *Eucalyptus globulus* (Wendler *et al.* 1995), *Pinus radiata* (Fife & Nambiar 1984), and *Picea sitchensis* (Millard & Proe 1993), where spring remobilisation from overwintering leaves was independent of senescence.

Therefore, young trees of *N. fusca* were grown in sand culture during two annual cycles commencing with spring budburst, and were provided with different levels of N supply. During the first cycle all the N was enriched with ^{15}N , with N at natural abundance provided throughout the winter and during the second cycle. A series of destructive harvests were taken to establish, (a) whether N was stored in overwintering organs for remobilisation into new growth and, if so, the site of storage, (b) whether allocation of N to storage was limited to the growth period before winter dormancy in the first cycle, or whether it continued during winter, (c) whether remobilisation was limited to the early growth phase of peak N demand in

spring and, if so, whether it commenced before budburst, (d) the relative contributions of storage and fertiliser N to new growth in that period, and (e) whether these processes were influenced by variation in N supply.

3.2 MATERIALS AND METHODS

3.2.1 *Experimental design*

Young trees used in this experiment were the same trees used in the experiment described in Chapter 2, and both experiments proceeded together. The trees were supplied with three levels of nitrogen by irrigating the pots during an experimental period lasting for 518 days from 1 December 1996 to 2 May 1998, covering two successive annual cycles starting with budburst in spring. To identify nitrogen (N) acquired during the first cycle, N was supplied between 1 December 1996 and 3 July 1997 as $^{15}\text{NH}_4^{15}\text{NO}_3$ enriched to 5.54 atom %. Trees treated with high nitrogen (HN) each received a weekly total of 179.55 mg of ^{14}N and 16.2 mg of ^{15}N in three 0.25 litre (6 mM) applications. Trees treated with medium nitrogen (MN) each received a weekly total of 87.995 mg of ^{14}N and 8.1 mg of ^{15}N in three 0.25 litre (3 mM) applications. Low nitrogen (LN) pots received a weekly total of 14.961 mg of ^{14}N and 1.35 mg of ^{15}N in three 0.25 litre (0.5 mM) applications. For the remainder of the first cycle, from 3 July 1997 to budburst, and during the second cycle, nitrogen at natural abundance was supplied in equivalent quantities and concentrations. All other nutrients were as used by Millard & Proe (1993). Once the last application of labelled fertiliser had been given, pots were flushed through under gentle pressure from beneath with twice their volume of NH_4NO_3 at the appropriate treatment concentration, in a tank designed for the purpose. They were then watered intermittently through the day for a three week period (30 litres total per pot). This

treatment was designed to remove any residual ^{15}N from the pots, without disturbing the root systems of the trees.

3.2.2 Harvesting the trees

The sequence of harvests is shown in Fig. 3.1. There were no significant differences between treatments in either the date of commencement, or duration, of budburst. Budburst occurred first in MN trees (day 286), next in LN trees (day 289), and lastly in HN trees (day 291). Budburst durations were 38, 29 and 36 days respectively.

Six destructive harvests in all were made, two in the last part of the first cycle, in June (day 211) and September (day 283), and four within the second cycle, in October (day 321) and December (day 370) 1997, and in February (day 401) and April (day 501) 1998. The October harvest was timed to coincide with the approximate mid-period of budburst in all treatments. Six trees from each treatment were selected randomly for each harvest. New stem growth was identified using the position of internodes and stem colour. The trees were removed and separated into buds, leaves and branches. Roots were carefully removed from pots in order to retain all material, and washed. All tree components were freeze-dried, weighed dry, and milled. Harvested leaves fell into two classes. The first class consisted of healthy and fully functional leaves, described as “young leaves”. Leaves in the second class had become discoloured, were in course of abscission, and were described as “old leaves”. First and second cycle leaves were not distinguished in these classes, but it was assumed that all first cycle leaves fell during the second cycle.

To determine if there was any labelled N in the pots flushed following the change to unlabelling fertiliser, all sand from the October harvest pots was

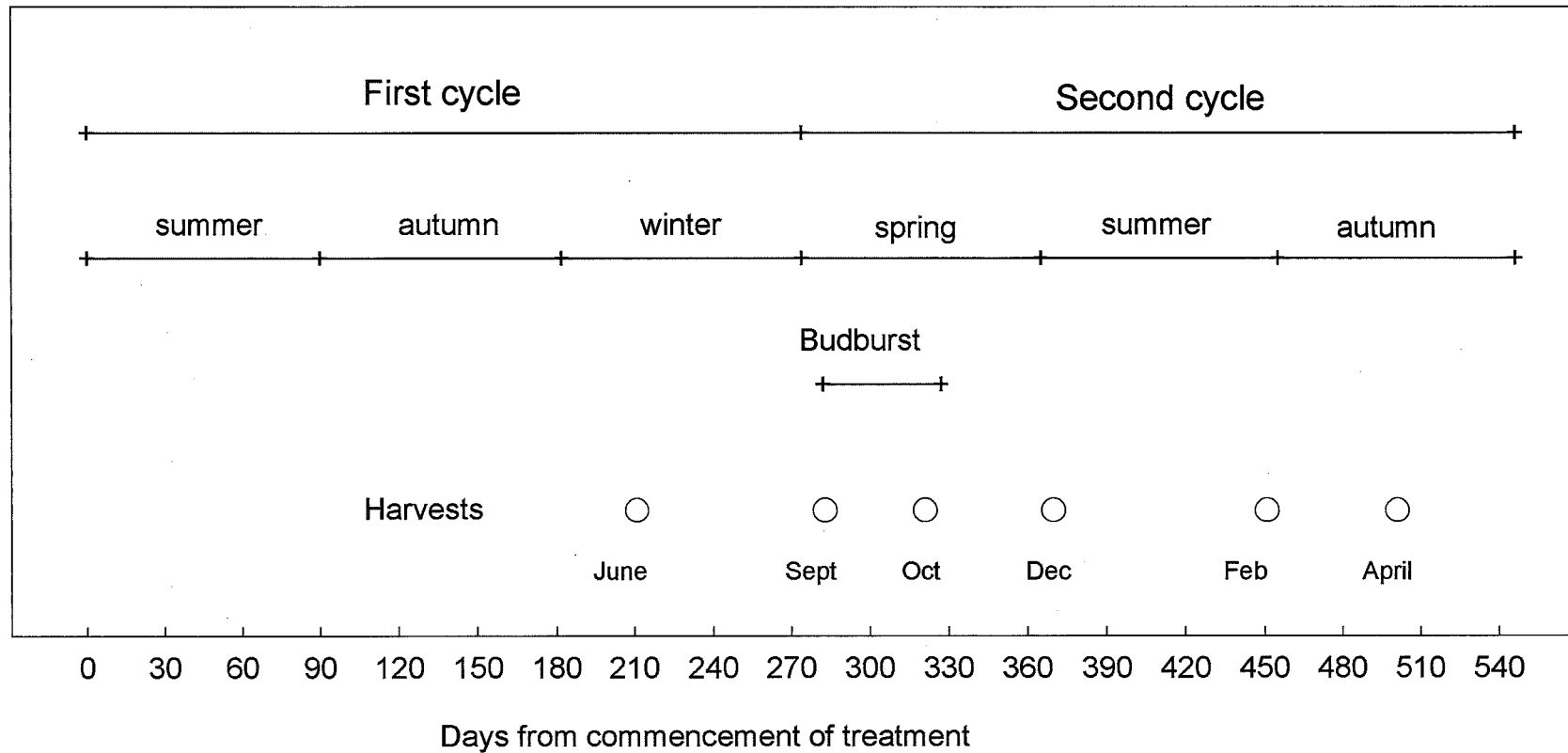


Figure 3.1. Diagram showing timing and duration of budburst and dates for harvests throughout the experimental period.

combined for each treatment and washed with water, the solution being freeze-dried for analysis. In addition, soil from the December harvest was returned to pots and three pumpkin seedlings germinated per pot, grown in shaded conditions in a glasshouse for 40 days without further irrigation, were then harvested, freeze-dried, combined (per pot) and milled.

Total N concentrations and ^{15}N abundances were determined by ANA-SIRA mass spectroscopy (VG Isogas, Middlewich, Cheshire, U.K.). ^{15}N enrichment was calculated by subtracting the background (determined from analysis of plants replaced in the rows, which received only non-enriched fertiliser), and was used to calculate the remobilisation of storage N as described in Millard & Nielsen (1989). Comparative calculation using abundances from analysis of both October pot soil extract and also the pumpkin seedlings grown in December harvest pots were used as background to calculate any error in results for October and December arising from any residual ^{15}N atom % excess in pots at those times.

3.2.3 Statistical analyses

Two way analysis of variance (ANOVA) was used to test for the main and interactive effects of time and treatment. Differences were considered significant if probabilities (P) were less than 0.05. Where ANOVA assumptions were met marginally, probabilities less than 0.01 were required. In all cases, log or square root transformation was necessary before undertaking the ANOVA and means and standard errors are reported after retransformation, with assymetric error bars calculated from mean standard errors (MSE) (Maindonald & Cox, 1984; Fowler & Cohen 1992). Comparisons by t test were made assuming unequal variances.

3.3 RESULTS

3.3.1 *Growth enhancement*

The nitrogen treatment had an effect on tree biomass (Fig. 3.2). Mean total dry weight of MN trees at the final harvest was 12 times greater than for LN trees ($P < 0.001$). However, doubling the N supply to the HN level did not increase this, and there was no significant difference between the final total dry weights of HN and MN trees (Fig. 3.2a). Mean dry weight of leaves for MN trees was 10 times greater than for LN trees ($P < 0.001$), but there was a trend for greater weight in HN trees (Fig. 3.2b). Mean stem dry weight was 16 times greater for MN trees than for LN trees ($P < 0.001$) (Fig. 3.2c) but, again, significantly greater weights did not result from doubling N supply to HN levels. Mean root dry weight was 18 times greater for MN trees than for LN trees ($P < 0.001$) but only half this for HN trees. The reduction of root dry weight of HN trees between February and April (Fig. 3.2d) was not significant.

There were 40% and 67% reductions in mean root dry weights of MN and HN trees midway through the budburst period (day 321), again as a trend (Fig 3.2d).

3.3.2 *Plant nitrogen content*

Overall uptake by trees of labelled N applied to the sand during the labelling period was low in all treatments and, measured at the September harvest (day 283), was 3% of labelled N applied for HN trees, 4.4% for MN plants and 2.7% for LN trees. All trees, irrespective of treatment, contained small amounts of unlabelled N at the first harvest in June (day 211)(Figs. 3.3a-c), by which time trees were dormant. This unlabelled N represented N acquired by trees before the labelling period.

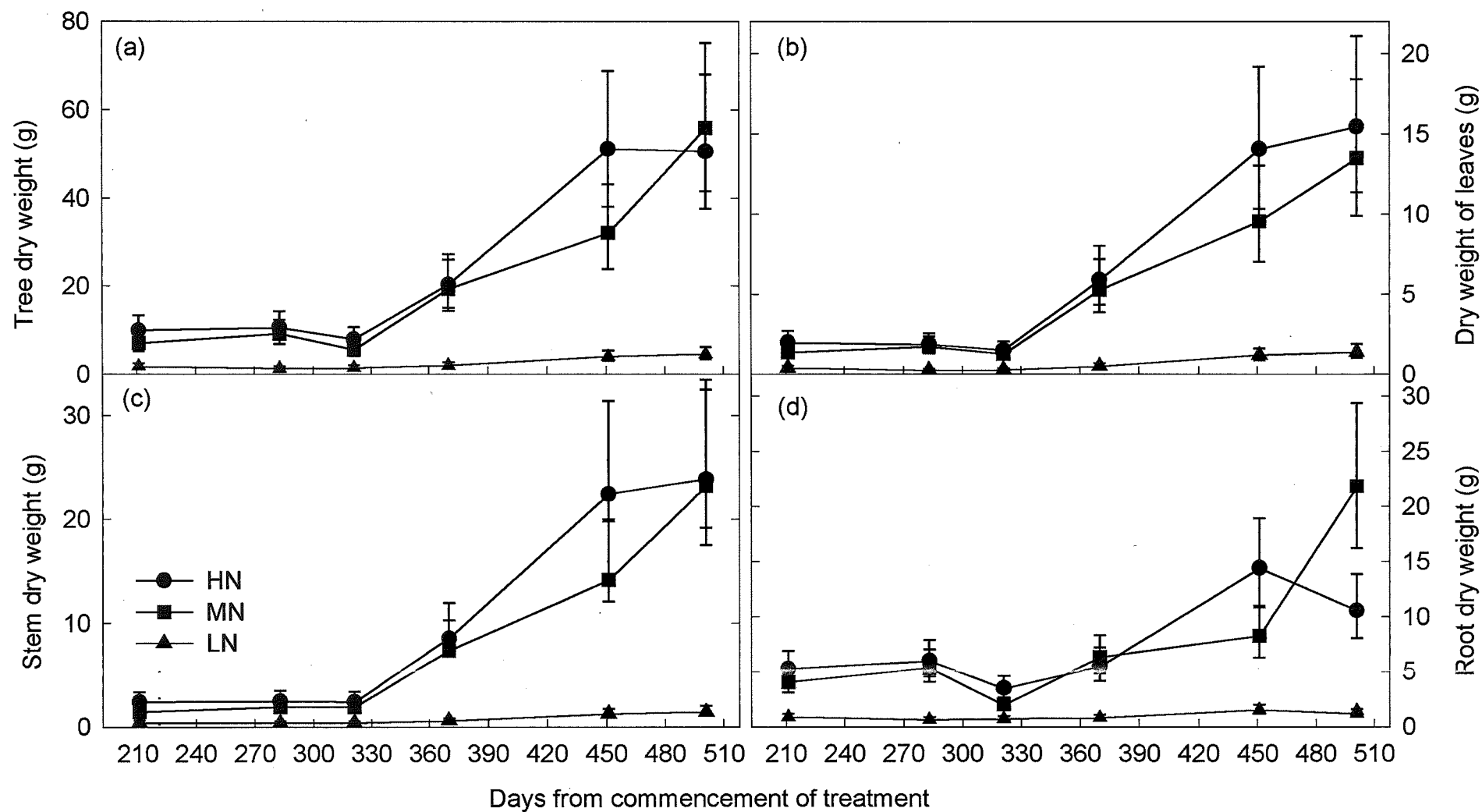


Figure 3.2. Changes in (a) mean whole tree dry weights (b) mean dry weights of all leaves (c) mean stem dry weights, and (d) mean root dry weights of *Nothofagus fusca* trees grown at three different concentrations of nitrogen supply (HN = high, MN = medium, LN = low). Error bars are \pm SE.

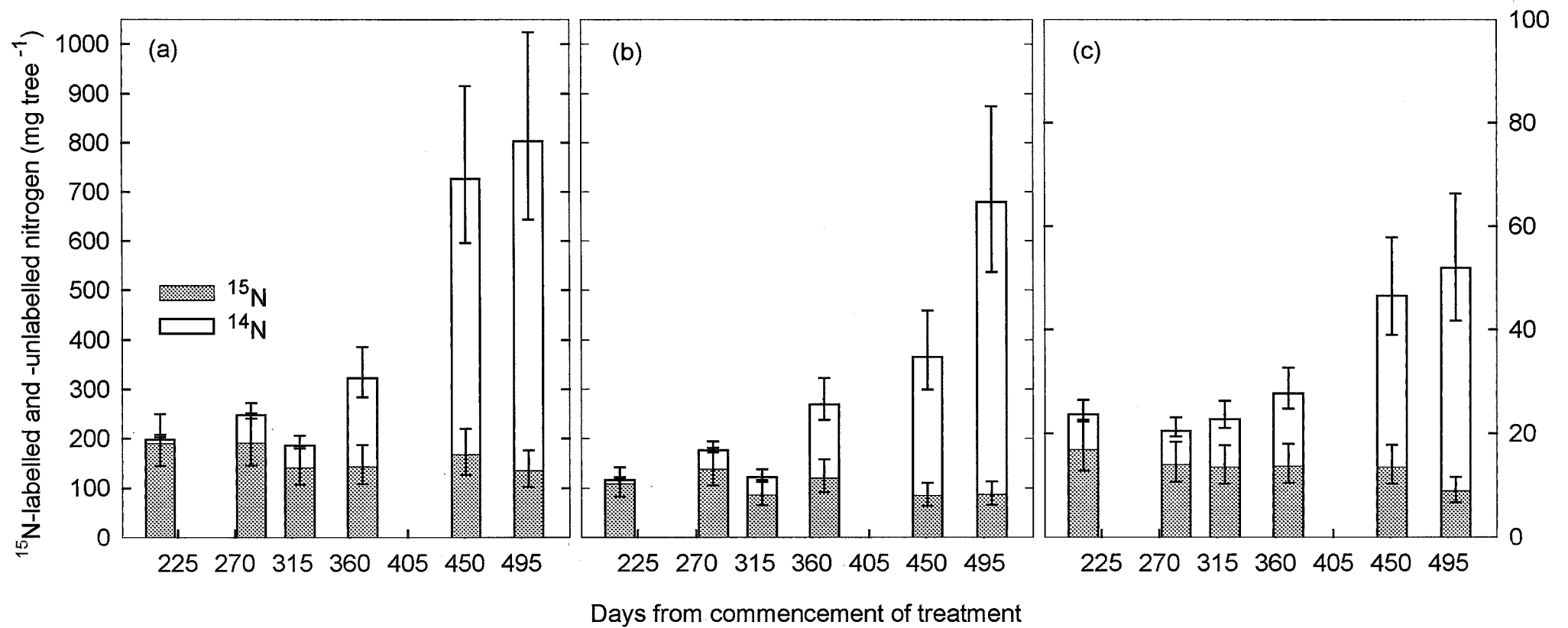


Figure 3.3. Changes in mean quantities of ^{15}N -labelled and unlabelled nitrogen in *Nothofagus fusca* trees grown at three different concentrations of nitrogen supply, (a) high nitrogen, (b) medium nitrogen, and (c) low nitrogen. Error bars are \pm SE.

Quantities of both labelled and unlabelled N in the trees at each harvest are shown in Figs. 3.3a-c. There were treatment effects on the amounts of total, and unlabelled N present in trees at the last harvest. Quantities were respectively 14 and 13 times greater for MN trees than for LN trees ($P < 0.001$). While differences were not significant, there was a trend for these amounts to be 15 times greater in HN trees than in MN trees (Fig. 3.3). There was also a treatment effect on the amounts of labelled N in trees at spring (September, day 283) (Fig. 3.3). Quantities were 14 times and 10 times greater in HN and MN trees, than in LN trees ($P < 0.001$). The difference between HN and MN values was not significant. There was little loss of labelled N from trees after spring, except as a trend towards the end of the experiment period (Figs. 3.3a,b,c). Labelled N comprised almost two thirds of total in HN trees at the start of summer (day 370) (Fig 3.3a), and approximately one third in both MN and LN trees (Fig. 3.3b,c).

3.3.3 Remobilised N in new tissue

New leaf, but not stem, growth was present in October (day 321). The simultaneous use of both labelled (remobilised) and unlabelled N for leaf growth is shown in Figs. 3.4 a,b., and for stem growth, in Figs. 3.4c,d. Quantities were always lowest in LN trees ($P < 0.001$). Following budburst, labelled N appeared in young leaves and stem (Figs. 3.4a,c), and peak quantities in young leaves were reached by early summer (day 370) ($P < 0.001$) (Fig. 3.4a). By early summer, remobilised N contributed 41.4%, 37% and 43.9% to total N in new leaves of HN, MN and LN trees, respectively, and 40.4%, 34.6% and 44.4% to total N in new stem (Figs. 3.4a,c).

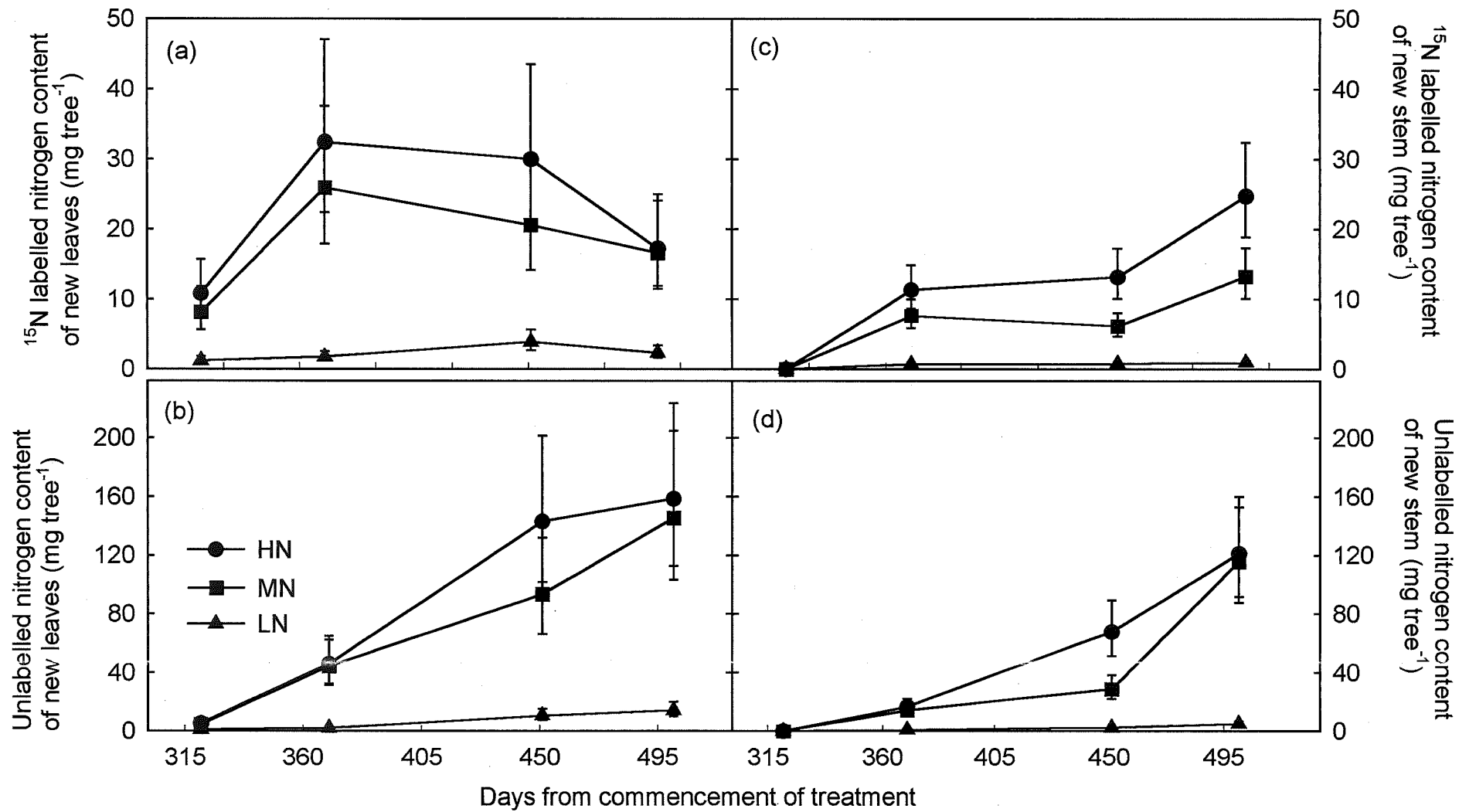


Figure 3.4. Changes in mean quantities of (a) ^{15}N -labelled nitrogen in young leaves (b) unlabelled nitrogen in young leaves (c) ^{15}N -labelled nitrogen in young stems (d) unlabelled nitrogen in young stems of *Nothofagus fusca* trees grown at three different concentrations of nitrogen supply (HN = high, MN = medium, LN = low). Error bars are \pm SE.

3.3.4 Site and amounts of N storage during winter

There were no significant reductions of labelled N from old leaves and stems following budburst (Figs. 3.5a,b)($P < 0.05$).

In contrast, N was stored during the winter in roots (Fig. 3.5c). Remobilisation commenced before budburst in all treatments and ended by the start of summer in HN and MN trees (Fig. 3.5c). There was a reduction in the labelled N content of roots between September (day 283) and October (day 321) or December (day 370) in both HN and MN trees. This was not evident in LN plants, which showed a steady reduction between June 1997 and April 1998. There were no time*treatment interactions but there was a time effect between June (day 211)($P < 0.001$) or September (day 283)($P < 0.002$) and April (day 501).

The amounts of stored N were calculated from the loss of labelled N from roots in spring (Fig. 3.5c). MN trees stored 20 times more than LN trees ($P < 0.001$), and so the quantity stored increased with N supply. Although the difference with MN trees was not significant ($P < 0.05$), there was a clear trend for greater storage in HN trees, 34 times more than for LN trees (Fig. 3.5c). By December (day 370), remobilised N represented 62.8%, 60.6% and 25.7% of total labelled N in roots of HN, MN and LN trees, respectively, in September (day 283). At the same time labelled N in new leaves and stem represented 64.7%, 85.86%, and probably all, of the N remobilised from roots of HN, MN and LN trees, respectively (Figs. 3.4a,b,c,d & 3.5c). A comparison with LN trees could not be made because calculated quantities of labelled N in young leaves and stems exceeded losses from roots at September, October and December harvests. However, between September and April, labelled N in roots of LN trees showed a gradual decline from 7 mg by a total of 3.5 mg (Fig. 3.5c), when 3.2 mg was found in young leaves and stems (Fig.

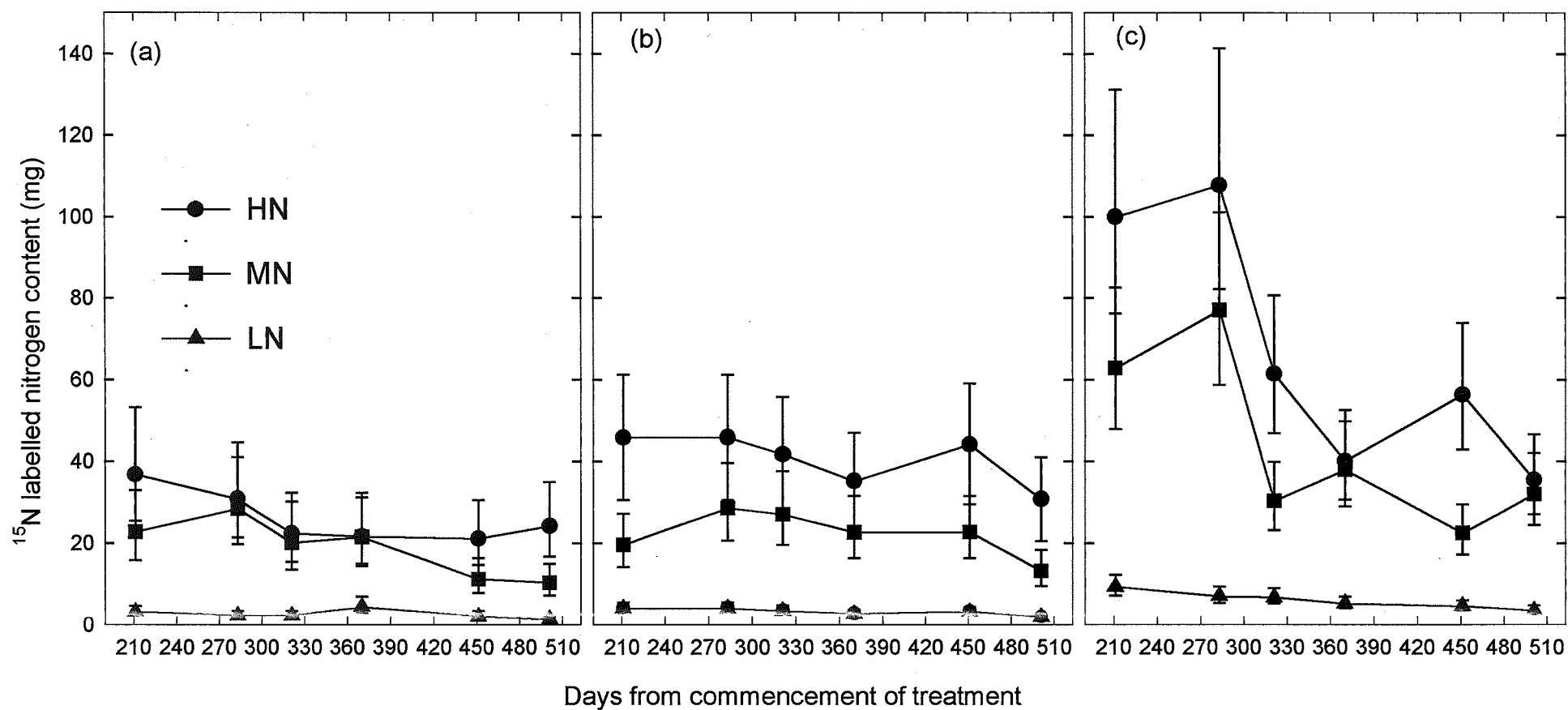


Figure 3.5. Changes in mean quantities of ^{15}N -labelled nitrogen in (a) old leaves (b) old stems, and (c) roots, of *Nothofagus fusca* trees grown at three different concentrations of nitrogen supply, (HN = high, MN = medium, LN = low). Error bars are \pm SE.

3.4a,c). This suggested a gradual movement of N from roots to shoots over the entire growing period of the second cycle in that treatment.

There was also evidence in HN and MN trees (but not LN trees) of continuing uptake of unlabelled N during the winter dormancy period 4 July (day 215) to September (day 283), when the small quantities present in June (day 211) increased 7 times, and 5 times, respectively ($P < 0.04$) (Fig. 3.3a,b). Storage of N from root uptake during winter was primarily in the roots (Table 3.1).

3.3.5 Background ^{15}N atom % excess

Compared with ^{15}N abundance of labelling fertiliser (5.539 atom %) analysis of the October sand solutions showed reductions to 0.694 (HN), 0.998 (MN) and 0.907 atom % (LN). ^{15}N abundances determined from analysis of pumpkin seedlings grown in December harvest soils showed even greater reductions. It was assumed that, in all cases, quantities of unlabelled N available in plant pools in September would be remobilised to new leaves and stem in the same proportions as labelled N. On this basis, possible small errors in the reported quantities of labelled or unlabelled N in October calculated using ^{15}N labelling alone, were reduced or negated, and were insignificant, being 2.53 mg (HN), 2.17 mg (MN) and 0.55 mg (LN) for young leaves, and nil (in each case) for new stems.

Table 3.1. Uptake of nitrogen during winter, and storage sites, as shown by increases in mean ^{14}N content between June and September, in young trees of *N. fusca* grown at three different levels of nitrogen concentration (HN = high nitrogen; MN = medium nitrogen; LN = low nitrogen).

| Nitrogen content | | ^{15}N (mg) | ^{15}N (mg) | ^{14}N (mg) | ^{14}N (mg) |
|------------------|--------|----------------------|----------------------|----------------------|----------------------|
| Treatment | Tissue | June | September | June | September |
| HN | Roots | 100.00 (29.90) a | 107.77 (25.62) a | 3.61 (1.06) b | 39.70 (10.09) c |
| | Stem | 47.49 (9.74) a | 49.12 (11.68) a | 1.99 (0.38) b | 9.07 (2.24) c |
| | Leaves | 36.83 (7.62) a | 30.82 (7.96) a | 2.01 (0.36) b | 5.98 (1.72) c |
| | Buds | 5.92 (1.09) a | 5.09 (0.79) a | 0.24 (0.04) b | 0.63 (0.04) c |
| MN | Roots | 62.90 (17.40) a | 76.91 (26.28) a | 4.21 (1.45) b | 26.88 (9.77) c |
| | Stem | 20.73 (4.35) a | 30.43 (10.99) a | 1.55 (0.37) b | 6.07 (2.13) c |
| | Leaves | 22.77 (5.38) a | 28.41 (10.92) a | 1.93 (0.38) b | 5.61 (2.07) c |
| | Buds | 1.72 (0.44) a | 2.40 (0.75) a | 0.12 (0.03) b | 0.34 (0.1) c |
| LN | Roots | 9.29 (1.39) a | 7.04 (1.31) a | 3.09 (0.41) b | 3.21 (0.58) b |
| | Stem | 4.23 (0.51) a | 4.33 (0.94) a | 1.74 (0.2) b | 2.00 (0.32) b |
| | Leaves | 3.16 (0.46) a | 2.25 (0.40) a | 1.81 (0.21) b | 1.14 (0.26) b |
| | Buds | 0.28 (0.05) a | 0.41 (0.09) a | 0.11 (0.02) b | 0.14 (0.02) b |

Figures in brackets are (\pm SE). Significant differences between values within each row, are indicated by different letters ($P < 0.05$).

3.4 DISCUSSION

Growth of young *N. fusca* trees was enhanced by increasing the N supply (Fig. 3.2). However, when N supply was doubled from the MN level there was no further increase in whole tree biomass, stems or roots of HN trees, although there was a trend towards greater leaf biomass (Figs. 3.2a-d). A possible explanation is that, in the absence of changes in photosynthetic capacity of individual leaves (Chapter 2), the increase in N supply stimulated foliage growth beyond an optimum, at the expense of stem growth (Brix & Ebell, 1969).

N was remobilised from storage to support the initial growth of leaves and stem growth during spring of the second cycle. However, there was also simultaneous uptake of N by the roots (Fig. 3.4), similar to that reported for deciduous *Acer pseudoplatanus* (Millard & Proe, 1991) and *Betula pendula* (Wendler & Millard, 1996). The proportion of labelled (remobilised) N to total in young leaves (Figs. 3.4a,b) and stems (Figs. 3.4c,d) from all treatments was highest at the start of summer, in December (day 370), being about 40% of total. This showed that all trees relied on root uptake for more than half of the N required for early growth. Simultaneous root N-uptake and N-remobilisation from storage was only defined between the start and end of spring, because of measurement intervals. More frequent sampling was required to confirm this (see Chapter 4).

N remobilised for growth of new leaves and stem of young *N. fusca* trees during spring, was stored during winter in roots (Fig. 3.5c), as reported for young deciduous apple and peach (Taylor & May 1967; Tromp 1983), *Acer pseudoplatanus* (Millard & Proe 1991) and *Betula pendula* (Wendler & Millard 1996). Root storage was not, however, present to any appreciable extent in mature evergreen citrus (Feigenbaum et al. 1987) or *Eucalyptus globulus* seedlings (Wendler et al. 1995).

This suggests that N storage in roots of young *N. fusca* trees, which is an evergreen species, follows a pattern reported in young northern hemisphere deciduous species.

The change to non-labelling fertiliser was made when all trees were dormant in early winter. Winter uptake of N has been reported previously in evergreen *Eucalyptus globulus* seedlings in Portugal, where minimum day/night temperatures were 12/10 °C (Wendler *et al.* 1995). Photosynthesis at reduced rates during winter might provide energy required for N uptake and storage. In this study, HN and MN trees continued taking up N during winter. This did not apply to LN trees and so winter uptake seems related to N supply levels. Winter uptake represented approximately 20% of all N taken up by HN and MN trees during the first cycle following the commencement of treatments (Figs. 3.3a,b). Since there was no growth during this period, it must have been taken into storage. The amounts taken up were equivalent to 53% and 60%, respectively, of the labelled N remobilised in spring by those trees. Winter uptake must therefore be considered as an important storage process in this species.

Trees withdraw N from senescing roots (Persson 1979; Ferrier & Alexander 1991), and the dip in HN and MN root weights between September and December harvests (Fig. 3.2 d), although not significant ($P < 0.05$) is, at least, not inconsistent with remobilisation accompanied by fine root turnover during that interval. This decline in root weights (between days 283 and 370) also coincided with the emergence of new leaves at budburst (day 321), and with a period of high proportional loss of old first cycle leaves (days 289 to 366) (Chapter 2). During this period a half and a third, respectively, of all leaves on HN and MN trees were shed and hydraulic demands were reduced. This spring pulse of leaf loss is seasonal in *N. fusca* (Bussell 1968), and is considered to be a deciduous characteristic in an

otherwise evergreen species (Wardle 1984). The discrepancy between the amount of labelled N recovered in new leaves and stem in early summer, and the amount of labelled N lost from roots over the same period, was considerable, and represented about 30% of labelled N lost from roots (Figs. 3.4 & 3.5). This was also consistent with fine root turnover.

In conclusion, young *N. fusca* trees used in this study responded to increased N supply by increasing total photosynthetic surface area, without much change in the photosynthetic capacity of individual leaves (Chapter 2). This response in young trees is consistent with adaptation to suppression beneath an essentially evergreen overstorey (Walters *et al.* 1993; Walters & Reich 1996, 1997). *N. fusca* also tends to inhabit river terraces and the mid-slopes of inland valleys on deeper, more fertile soils (Ogden, Stewart & Allen, 1996), and foliar analysis suggests it has higher nutrient requirements (Adams, 1976). This implied some sensitivity to soil nutrient status, and also an important role for site nitrogen availability, in competition between young trees in the understorey or upon release through a canopy gap. Root storage of N, possibly involving seasonal fine root turnover, provides another factor in understorey competition for nutrients, due to sub-soil spatial interactions (Grace 1995), and the relationships between micro-site nitrogen and water availabilities, and root volume (Wendler & Millard 1996; Walters & Reish 1997).

3.5 SUMMARY

Increasing the N-supply resulted in increased growth of young *N. fusca* trees. Processes of N-uptake, internal storage, and remobilisation of storage-N, were not changed by manipulating tree N-status. Depending on N-supply, trees continued

taking up N for storage during winter. The site of N-storage was uniformly in roots, and approximately 40% of all N in new stem and leaves in spring came from storage. The storage site in roots contrasted with storage in leaves of evergreen trees. It was similar to root storage in young winter-deciduous trees from the Northern Hemisphere. Simultaneous root N-uptake and N-remobilisation from storage was only defined between the start and end of spring, because of measurement intervals. More frequent sampling was required to confirm this, and this was undertaken in the experiment reported in the next chapter. Seasonal timing of N-storage and remobilisation was synchronous with patterns of timing of growth and loss of leaves reported in Chapter 2. The implications of these processes as a strategy for meeting seasonal demand for C and N for growth, are discussed in Chapter 5.

CHAPTER 4

EFFECTS OF CARBON SUPPLY ON PHENOLOGY AND TIMING OF SOIL NITROGEN UPTAKE IN JUVENILE TREES OF *NOTHOFAGUS FUSCA* (HOOK. F.) OERST.

4.1 INTRODUCTION

Nothofagus fusca exhibits the evergreen habit of maintaining a canopy of leaves year round. However, the species also shows loss of old leaves in spring when new leaves emerge and grow from over-wintering buds, set in the previous season (Russell, 1936; Bussell, 1968). These phenological events influence the timing, and quantity, of new seasonal leaf area (Chapter 2). They also affect the timing, and magnitude, of peak demand for carbon (C), and nitrogen (N) acquisition. Young trees of *N. fusca* remobilise N from storage in the roots, and contemporaneously take up N from the soil (Chapter 3). Changes in the timing of these phenological events can be expected, therefore, to produce changes in the timing of demand for C and N.

Growth in an environment of elevated CO₂ concentration, has been known to produce changes in the timing of budburst, and also bud mass, in some tree species. Budburst was delayed in both *Picea sitchensis* (Lee, Barton & Jarvis 1993), and *Castanea sativa* (El Kohen, Venet & Mousseau 1993). The dry weight of buds of *Fagus sylvatica* was increased (Overdiek 1993). Enhanced C supply also increased the initial rate of new leaf expansion in four *Eucalyptus* species (Wong, Kriedeman & Farquhar 1992), and the overall leaf expansion rate of young *Populus euroamericana* (Gaudillere & Mousseau 1989). The growth rhythm of stem

extension, (proportional growth with time), was affected in *Salix phylicifolia*, where a high CO₂/nutrient ratio lead to a shorter growing period (Silvola & Ahlholm, 1993). Increases in the numbers of growth flushes, and secondary branches and leaves, and in the size of individual leaves, have also been reported. Growth of new secondary branches was seen in *Citrus aurantium* (Idso, Kimball & Allen 1991) and in *Fagus sylvatica* (El Kohen, Venet & Mousseau 1993), where it was accompanied by enlarged area of individual leaves. The dry weight of buds of *Quercus alba* was increased (Norby, O'Neill & Luxmore, 1986). Growth at elevated CO₂ concentration has been known also to influence the timing and pattern of leaf loss. Leaf senescence and abscission were advanced in *Castanea sativa* (Mousseau & Enoch 1989).

Carbon mediated changes in tree biomass depend on an interaction with plant N status. Using meta-analysis to synthesise research results from many studies, Curtis (1996) and Curtis & Wang (1998) found that biomass responses to enhanced C supply were strongly affected by environmental stress factors. Plants grown under nutrient stress showed only half the percentage growth stimulation of plants with no stress treatment. If N and C supply rates are commensurate, the result is usually that trees grow larger more quickly, with little change in biomass and physiology. If N is in short supply, there may be developmental and physiological changes. Dry mass allocation to roots, phenology, and photosynthesis may be altered (Jarvis 1995). Elevated CO₂ concentration has the effect of reducing tree N status, unless trees are well fertilised. Using *Nicotiana tabacum*, Geiger *et al.* (1999) demonstrated that elevated CO₂ leads to a decrease in the levels of key metabolites in N metabolism when plants are not supplied with optimal levels of N, producing a reduction in nitrogen status. Even at intermediate levels of supply, the reduced N status may just

suffice to avoid N limitations at ambient CO₂ concentrations (see Chapter 1). Similar results were reported for *Pinus ponderosa* (Johnson *et al.* 1995), and *Gossypium hirsutum* (Rogers *et al.* 1996).

The effects of C supply on phenology are important because the timing of budburst is a critical determinant when modelling ecosystem production (Myerani *et al.* 1997; Hakkinen, Linkosalo & Hari, 1998). In young trees of *N. fusca*, the onset and duration of budburst were not affected by differences in N status (Chapters 2 and 3), although bud numbers and bud mass were greatly increased with enhanced N supply. This gave rise to the question of whether, in trees with a common N supply, the onset of budburst, and its duration, are affected by C supply. Also, young trees of *N. fusca* rely simultaneously on storage N, and N from root uptake, to meet the N requirements for new growth following budburst (Chapter 3). If the onset and duration of budburst were altered by C supply, this could result in changes in the timing of peak demand for N for new growth and changes in the timing of root uptake of N relative to remobilisation of N from storage.

Juvenile *N. fusca* trees have been growing at ambient and elevated CO₂ concentration in open-top chambers at Bromley, Christchurch, New Zealand, for five years (Whitehead *et al.* 1995). These trees were used in this study to test the following hypotheses:

- (a) Budburst, and hence peak demand for N and C, would be advanced by increased C supply.
- (b) Root uptake of N would lag behind remobilisation of N from storage.
- (c) An earlier peak demand for N would result in earlier leaf senescence and abscission.

Here the approach was to use the elevated CO₂ concentration to alter the C/N relations of experimental trees to probe patterns of phenology and nitrogen utilisation in *N. fusca*.

4.2 MATERIALS AND METHODS

4.2.1 Plant material

Tree seedlings of *Nothofagus fusca* (Hook f.) Oerst, collected from natural *Nothofagus* (beech) forest near Maruia Springs, (42° 12' S, 172° 15' E) (Hogan *et al.* 1996), were grown in large open-top chambers at Christchurch, New Zealand, as described in Whitehead *et al.* (1995), for five years. During this period three chambers were continuously supplied with CO₂ at a concentration of approximately 360 µmol mol⁻¹ ("ambient CO₂"), and three with an elevated concentration of approximately 650 µmol mol⁻¹ ("elevated CO₂"). The site is flat and the soil is recently stabilised Kairaki dune sand, which is free to rapidly draining (New Zealand Soil Bureau 1974). This soil type has a typically low nitrogen content, 0.064% having been reported for the uppermost 100 mm horizon, and 0.019% for the next 100 mm horizon (Thomas 1987). Before the seedlings were planted 50 mm of natural beech soil was placed on the surface of the soil in the chambers (Whitehead *et al.* 1995). The mean number of trees receiving ambient CO₂ at the time of measurements (August 1998-March 1999) was 12 (± 1) for the three chambers receiving ambient CO₂ concentration and, was 11 (± 1) for the three chambers receiving elevated CO₂. The height, and mean diameter (at soil surface), of each tree were measured in August 1998. There were no significant differences between

chambers or treatments. Height of trees approximated 3800 mm, and stem basal area approximated 1300 mm².

4.2.2 Application of labelling fertiliser

All surface weeds and dead leaves were carefully removed from each chamber. To identify nitrogen (N) uptake from the soil, N was supplied as ¹⁵NH₄¹⁵NO₃ enriched to 10 atom %, with a dressing of 30.9 grams per chamber (1.8 g. m⁻²) on 23 September 1998, and a similar dressing on 24 September 1998. The soil was then covered with polythene to prevent leaching from rainfall.

4.2.3 Collection of soil samples and measurement of N content

Soil was sampled for N concentration to a depth of 110 mm and 220 mm. from three equi-distant points within the chamber. Soil was sampled immediately before fertiliser application, and again 8, 21 and 43 days later. N was extracted using 50 ml of 1M KCl per 25 g of sample, and sub-samples of the resulting solution were measured for total N content using an Elemental Analyser (Carlo Erba, Milan, Italy).

4.2.4 Budburst and leaf fall

Observations were made every three days during the experimental period from 23 September 1998 to 14 December 1998. Following fertiliser application the dates of initial and final budburst were recorded for each tree in each chamber. Three trees were selected randomly in each chamber ("sample trees"), and the canopy was divided into upper, central and lower portions. Initial budburst was invariably within the central third of the canopy. For each tree, three branches ("sample branches")

were selected randomly within the central third of the canopy, one to the south-west, one to the north, and one to the east. Dates of first and last budburst on these branches were recorded. All buds and leaves were counted for approximately 300 mm along the distal end of the southwest branch ("monitored branch"), and numbers of buds burst, and leaves fallen, were recorded.

4.2.5 Collection of bud and leaf samples

Bud or leaf samples were collected from the central third of the canopy. These were freeze-dried, counted and weighed before being milled to a fine powder. Following initial bud swelling and protrusion of leaf tips, (budburst), there was a delay of approximately 14 days (data not shown), following which, rapid emergence of new stem and leaves commenced. This applied equally for trees grown at both CO₂ concentrations. Buds collected at that point of time were dissected after being freeze-dried, and the number of leaves were counted and weighed. ¹⁵N abundance and ¹⁵N enrichment were determined as described in Chapter 3. In order to measure mean leaf areas and mass, and calculate specific leaf area, S , samples of sun leaves were collected from the top, central and lower canopy third's of each sample tree on 12 March 1999. Areas were obtained using a leaf area meter, then the leaves were oven-dried and weighed.

4.2.6 Analysis of leaf expansion rhythm

An average dry mass for an individual leaf from each tree was calculated for each collection after budburst. For each tree, the value obtained for each collection was normalised as a fraction of the greatest mass attained by a leaf from that tree. The seasonal increase in normalised values was related to the number of days that had

elapsed from budburst. The relationship was characterised using the Gompertz equation (Causton, 1977; Hunt, 1982), which was fitted by non-linear least squares procedure, as

$$M = M_m e^{-ce^{-dG}} \quad 4.1$$

where M_m is the maximum normalised value (i.e. 1), G is the number of days after budburst, and c and d are parameters.

4.2.7 Statistical analysis

Two way analysis of variance (ANOVA) was used to test for the main and interactive effects of chamber and treatment. Differences were considered significant if probabilities (P) were less than 0.05. Comparisons were made by t test, assuming unequal variances. Figures in brackets following mean values are mean standard errors.

4.3 RESULTS

Mean N content of the soil solutions in the upper 110 mm soil horizon obtained from all six chambers was about 5 mg L^{-1} before fertiliser application and, following fertiliser application, remained approximately twice that amount for at least 21 days, falling to about 4 mg L^{-1} after 43 days. Fine roots were mainly confined to the upper soil fraction. Mean N content of the lower 110 mm horizon followed a similar pattern, except that the amounts at the start and at 43 days were relatively greater.

The mean number of days from fertiliser application to budburst for all sample trees, was $17 (\pm 2)$. There were no significant differences between chambers or treatments in the commencement dates for budburst, or the duration of budburst. These were about 40 days from the start of spring, and 12-13 days, respectively

(Table 4.1). There was close agreement between all trees in the chambers, sample trees from each chamber, and sample branches on sample trees, in the timing of onset, and the duration, of budburst (Table 4.1). There was a trend for buds to burst initially in the central third of the canopy (Table 4.1). Buds on trees growing at ambient CO₂ concentration were more than 40% heavier ($P < 0.009$), and contained almost two-third's more nitrogen ($P < 0.018$), than those on trees growing at elevated CO₂ concentration (Table 4.2). The difference in nitrogen concentration mass based nitrogen concentration, N_m , of buds was not significant (Table 4.2) ($P < 0.05$). One foliage flush only, (in spring-early summer), was observed in the growing season from spring 1998 to 12 March 1999.

There was no significant difference in the mean number of days from budburst to full expansion of new leaves, in both cases being 27 days (Table 4.1). However, the pattern of increase of mass of individual leaves was different (Fig. 4.1). Seasonal increase was deferred for trees growing at elevated CO₂ concentration. There was no significant difference between chambers or treatments, in the commencement of leaf fall from monitored branches. Leaves began falling between five and six weeks after the start of spring (Table 4.1). There was a clear trend, demonstrated from monitored branches, for trees growing at elevated CO₂ concentration to retain leaves longer than that for trees growing at ambient CO₂ concentration. Almost all leaves had fallen from monitored branches on trees growing at ambient CO₂ concentration, 75 days after the first leaf fell, whereas a third were still retained by those experiencing elevated CO₂ (Fig 4.2).

Increase in the mass of individual leaves was similar in both treatments until four weeks after budburst but following this, the trend was for values to be relatively greater for trees growing at elevated CO₂ concentration (Fig. 4.3). At 42

Table 4.1. Mean values for the commencement and duration of budburst, the time taken for full expansion of new leaves, and the commencement of fall of old leaves, of trees of *N. fusca* grown at two different concentrations of CO₂, ambient and elevated, in large open-top chambers for five years.

| Measured variable | Elevated CO ₂ | Ambient CO ₂ |
|---|--------------------------|-------------------------|
| Mean number of days from start of spring to first budburst anywhere on tree. (All trees in chambers, three chambers per treatment) | 39 (\pm 1) n = 32 | 42 (\pm 2) n = 34 |
| Mean number of days from first to last budburst on tree. (All trees in chambers, three chambers per treatment) | 13 (\pm 1) | 12 (\pm 1) |
| Mean number of days from start of spring to first budburst anywhere on sample trees (three per chamber, three chambers per treatment) | 36 (\pm 2) | 43 (\pm 4) |
| Mean number of days between first and last budburst on sample trees | 20 (\pm 3) | 15 (\pm 3) |
| Mean number of days between first budburst anywhere on tree and first budburst on sampled branch (sample trees) | | |
| Tip | 8 (\pm 2) | 5 (\pm 1) |
| Centre | 3 (\pm 1) | 3 (\pm 0) |
| Base | 6 (\pm 2) | 5 (\pm 1) |
| Mean number of days between first and last budburst on sampled branches (sample trees) | | |
| Tip | 11 (\pm 2) | 8 (\pm 1) |
| Centre | 9 (\pm 1) | 9 (\pm 1) |
| Base | 7 (\pm 2) | 5 (\pm 1) |
| Mean number of days to full expansion of new leaves | 27 (\pm 2) | 27 (\pm 3) |
| Mean number of days from start of spring to first leaf fall from monitored branch (sample trees) | 40 (\pm 5) | 34 (\pm 2) |

Figures in brackets are \pm SEs. There were no significant differences between treatments ($P < 0.05$).

Table 4.2 Mean values, at budburst, for mass, total N content, ^{15}N -labelled N content, and bud nitrogen concentration (mass based), N_m , of buds of trees of *Nothofagus fusca* grown at two different concentrations of CO_2 , ambient and elevated, in large open-top chambers for five years.

| | Ambient CO_2 | Elevated CO_2 | |
|--|-------------------------|-------------------------|-------------|
| Mean mass (mg. bud $^{-1}$) | 5.88 (± 0.45) a | 4.16 (± 0.36) b | $P < 0.009$ |
| Total N content (mg. bud $^{-1}$) | 0.098 (± 0.011) a | 0.061 (± 0.008) b | $P < 0.018$ |
| Labelled N content (mg. bud $^{-1}$) | 0.010 (± 0.002) a | 0.004 (± 0.001) b | $P < 0.04$ |
| N_m (mg. g $^{-1}$) | 16.15 (± 0.92) a | 14.31 (± 0.74) a | N.S.D. |

Figures in brackets are \pm SEs. Significant differences along rows are indicated by different letters.

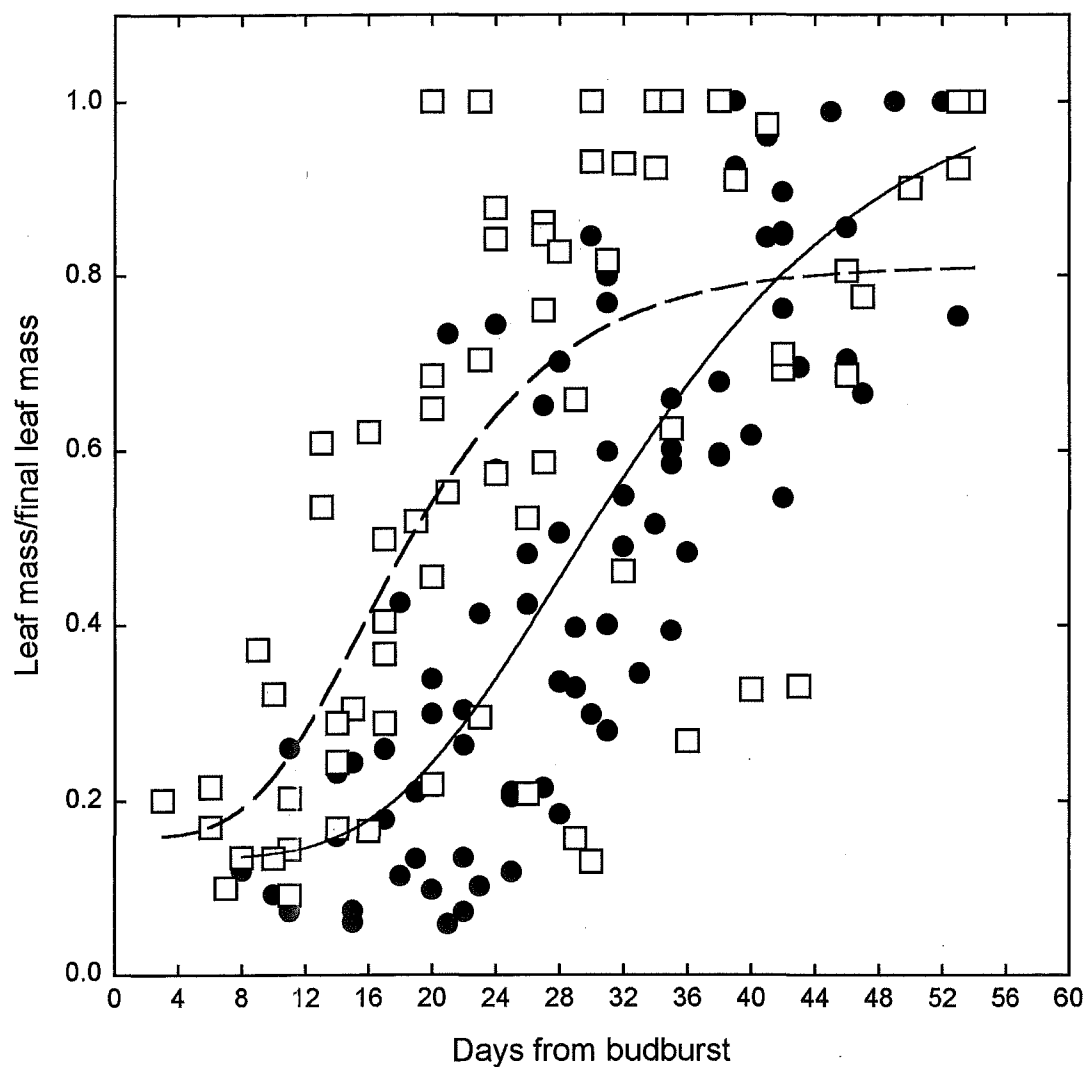


Figure 4.1 Growth rhythm (leaf mass/final leaf mass) of leaves of trees of *N. fusca* grown for 5 years at elevated (●) and ambient (□) CO₂ concentrations. The lines shown, elevated (—) and ambient (---) concentrations, were fitted using the Gompertz equation (Equation 4.1). $r = 0.82$ (elevated), and 0.69 (ambient).

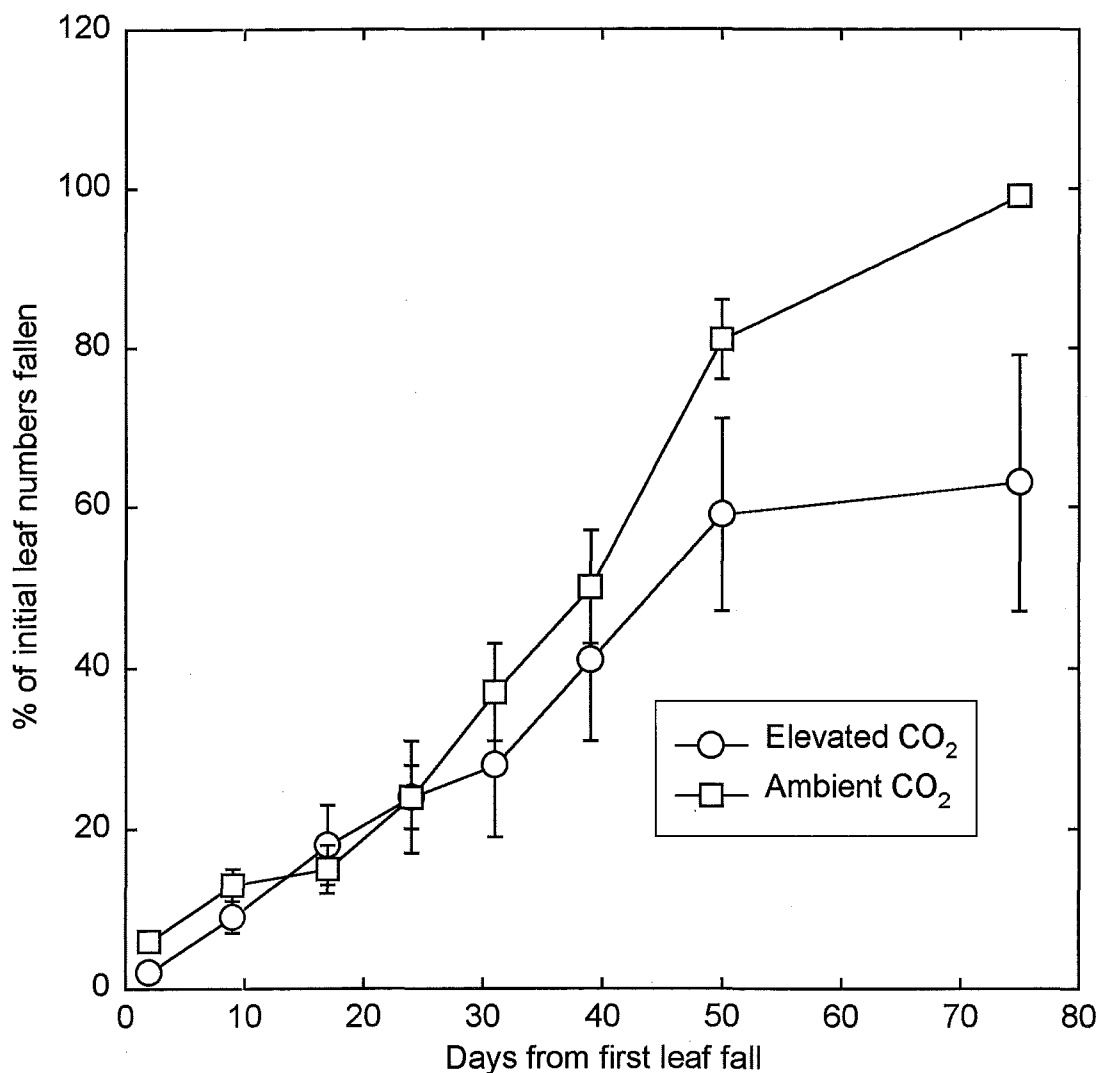


Figure 4.2 Mean number of leaves, as a percentage of the initial total, of leaves falling from monitored branches of trees of *N. fusca* grown at two different concentrations of CO₂, ambient and elevated, in large open-top chambers for 5 years. There were no significant differences at any point along the curves ($P < 0.05$)

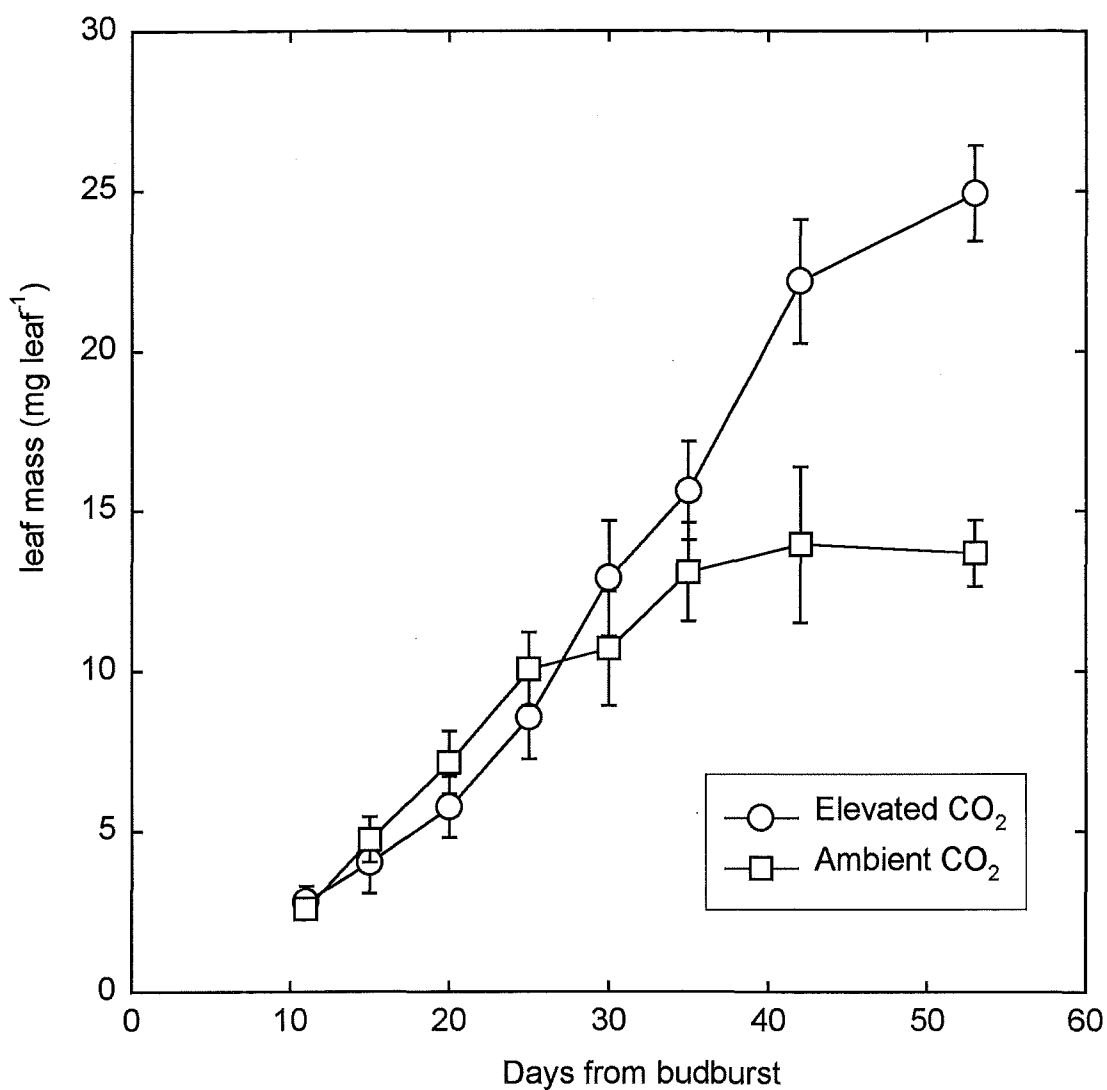


Figure 4.3 Increase in mass during expansion from budburst, of leaves of *N. fusca* trees grown for 5 years in ambient and elevated CO₂ concentrations.

and 53 days after budburst, these values were, respectively, about 50% and 80% greater than those at ambient CO₂ concentration (Fig. 4.3; $P < 0.02$, $P < 0.001$).

In both treatments, there was an initial increase in mass-based leaf N concentration, N_m , until the third week after budburst (Fig. 4.4). Then values began to decline. The trend during the first month following budburst was for values to be relatively greater in leaves of trees growing at elevated CO₂ concentration. After this however, values for that treatment declined relative to those for trees at ambient concentration. At 53 days after budburst, leaf N concentration (mass based), N_m , was significantly greater in leaves of trees experiencing ambient CO₂ ($P < 0.03$)(Fig. 4.4).

Total nitrogen content of individual leaves showed similar increase in both treatments, until approximately one month following budburst, when the trend was for a relatively greater increase in leaves of trees growing at elevated CO₂ concentration (Fig. 4.5). At 53 days after budburst, values were significantly greater for trees growing at elevated CO₂ concentration, than for those at ambient concentration ($P < 0.03$)(Fig. 4.5).

Mean leaf areas and weights of leaves at the beginning of autumn were significantly greater for trees growing at elevated CO₂ concentration ($P < 0.02$), but S was the same for both treatments, $90 \text{ m}^2 \cdot 10^{-4} \text{ g}^{-1}$, ($P < 0.001$)(Table 4.3).

All trees showed root uptake of labelled nitrogen had occurred by budburst (Table 4.2). There was an indication that mean quantities of labelled N, as a percentage of total N, might be lower in buds of trees receiving elevated CO₂ (6.0%, compared with 9.2% for trees receiving ambient CO₂), but the difference was not significant. This N uptake appeared to be continuous, for the entire 53 day period following budburst (Fig. 4.5).

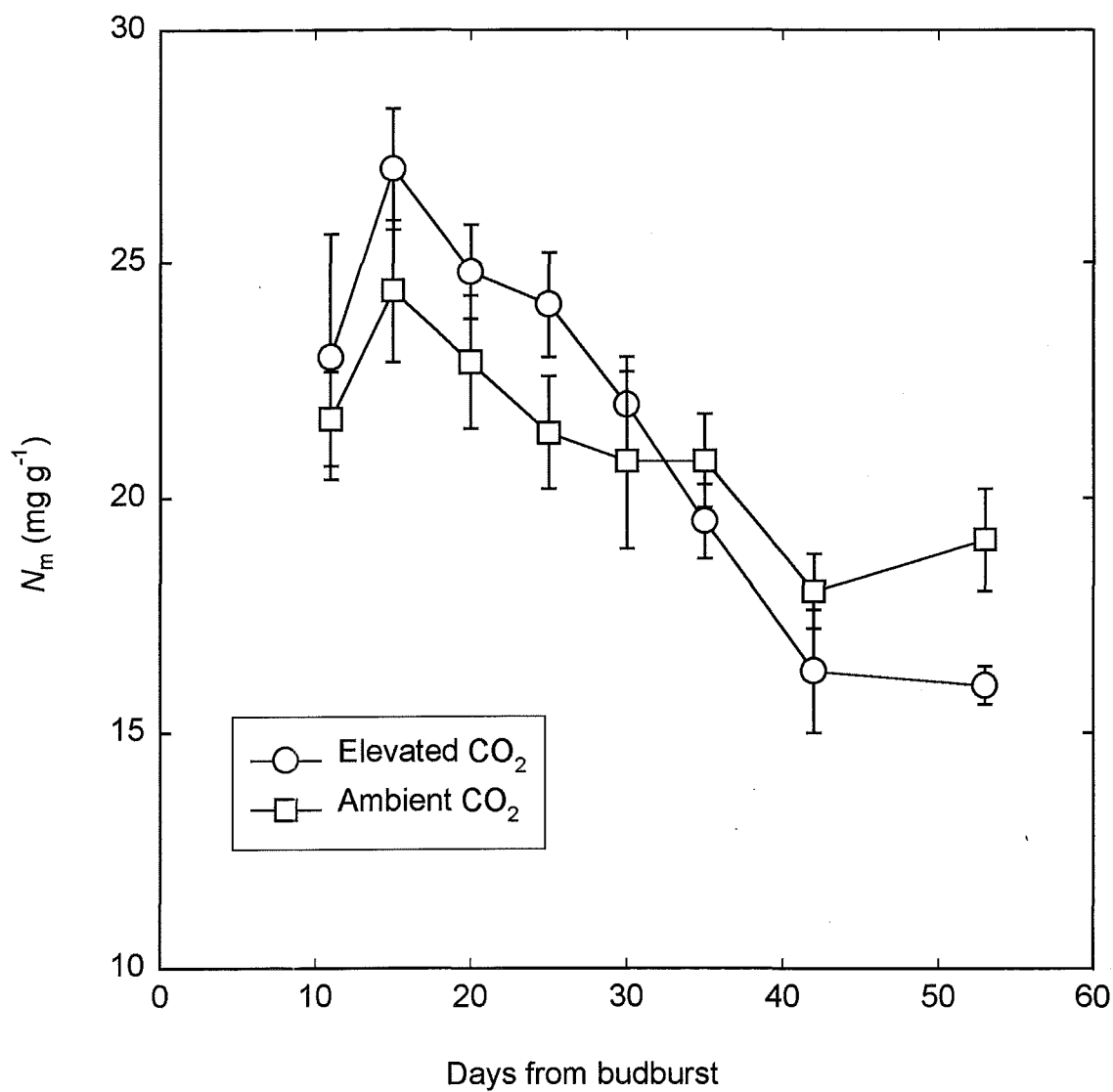


Figure 4.4 Changes in nitrogen concentration on a mass basis, N_m of leaves of *N. fusca* trees grown for 5 years at ambient and elevated CO_2 concentrations.

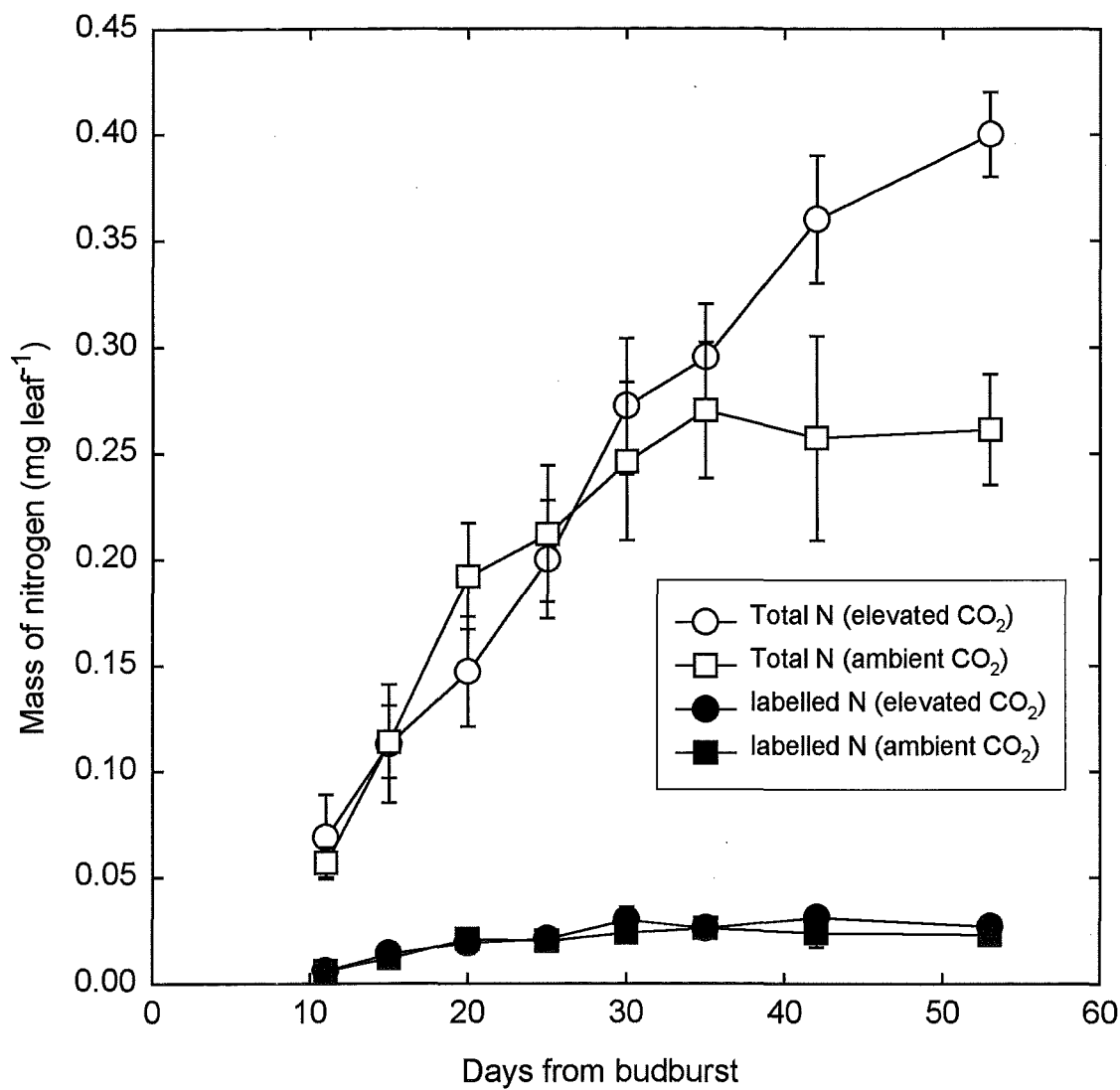


Figure 4.5 Increases in mass of total nitrogen, and labelled nitrogen in leaves of *N. fusca* trees grown for 5 years at ambient and elevated CO₂ concentrations.

Table 4.3. Mean leaf area, mass, and specific leaf area, S , of individual leaves, 193 days after spring (start of autumn), for trees of *N. fusca* grown at two different concentrations of CO₂, ambient and elevated, in large open-top chambers for five years, (three trees per chamber, three chambers per treatment)

| Measured variable | Elevated CO ₂ | Ambient CO ₂ |
|--|---------------------------|--------------------------------------|
| Leaf area (m ² .10 ⁻⁴ .leaf ⁻¹) | 2.2 (± 0.54) a n = 107 | 2.0 (± 0.52) b (P < 0.02) n = 108 |
| Leaf mass (g.10 ⁻³ .leaf ⁻¹) | 24.5 (± 0.54) a | 22.6 (± 0.63) b (P < 0.001) |
| Specific leaf area, S (m ² .10 ⁻⁴ .g ⁻¹) | 90 (± 1.0) a | 90 (± 1.0) a N.S. |

Figures in brackets are ± SEs. Significant differences between values within each row, are indicated by different letters (P < 0.05).

4.4 DISCUSSION

Juvenile trees of *N. fusca* did not show any changes in the timing of budburst as a consequence of growth at elevated CO₂ concentration (Table 4.1). This response differed from those reported for deciduous *Castanea sativa*, where budburst was delayed (El Kohen, Venet & Mousseau, 1993). In the present study, duration of budburst was also not affected nor was the time taken for new leaves to expand fully. These results are in contrast to deciduous *Populus euroamericana* seedlings, which showed increased leaf expansion rate with enhanced CO₂ (Gaudilliere & Mousseau, 1989). However, the delayed period of seasonal increase in the mass of individual leaves of trees growing at elevated CO₂ concentration (Fig. 4.1), was opposite to the response of *Salix phylicifolia*, where a high CO₂/N ratio led to a shorter period of seasonal increase (Silvola & Ahlholm 1993). The delay in seasonal increase, shown in the present study, may have been due to lower bud mass at budburst on trees growing at elevated CO₂ concentration (Tab. 4.1). This may also have resulted in lower mass (Fig. 4.3), and total nitrogen (Fig. 4.5) of individual leaves, during the first three weeks following budburst. The greater mass of individual buds on trees growing at ambient CO₂ concentration, contrasted with results from *Quercus alba*, where buds of trees growing at elevated concentration had greater mass (Norby, O'Neill & Luxmore (1986).

Only one flush of growth was observed in both C treatments during the period from budburst until early autumn. This contrasted with the response of *Fagus sylvatica*, which showed a greater number of growth flushes when grown at elevated CO₂ concentration (El Kohen, Venet & Mousseau, 1993).

There was no alteration in the timing of onset of leaf loss at budburst in response to manipulated plant C status. This contrasted with deciduous *Castanea*

sativa (Mousseau & Enoch, 1989) and *Betula pendula* (Evans & Lee, 1993), where senescence and abscission were advanced, producing a corresponding reduction in the growing season. However, the absence of such an effect in *N. fusca* may be due to differences in strategy between that species and these northern winter-deciduous species (see Chapter 5). *N. fusca* loses the balance of its previous season's leaves in a pulse *at the start* of the growing season, during a springtime-early summer process of replacement, so that it maintains a canopy in the manner of an evergreen species. Furthermore, to similar effect, it does not shed all its leaves at the end of the growing season, but retains a functional canopy of leaves during winter while the tree is growth-dormant (Chapter 2). In contrast, winter-deciduous *Castanea sativa* and *Betula pendula* shed all leaves *at the end* of the growing season and do not retain a functional canopy during winter quiescence. The consequence of early senescence and leaf fall in *Castanea sativa* and *Betula pendula* was a premature termination of canopy function, without any alteration to the overall strategy of a total absence of leaves during winter. The consequence of early senescence and leaf fall in *N. fusca* would be to alter the synchrony between pulsed leaf loss and replacement during the early phase of the growing season and defeat the strategy of always maintaining a functional canopy. Nor is leaf fall necessarily always advanced in deciduous trees. Senescence and abscission were unaffected in *Quercus alba* and *Liriodendron tulipifera* (Gunderson *et al.* 1993), although they were reportedly delayed in *Quercus alba* by Norby *et al.* (1986).

The clear trend for old leaves to be retained longer following budburst, on *N. fusca* trees growing at elevated CO₂ concentration (Fig. 4.2), tends to confirm the results from Chapter 3, where it was shown that leaves are not used as storage site for N remobilised for spring growth.

The larger individual leaf size on trees grown at elevated CO₂ concentration (Table 4.3), was seen also in deciduous *Fagus sylvatica*, although not in *Castanea sativa* (El Kohen, Venet & Mousseau, 1993). It was also seen in evergreen *Poncirus trifoliata* x *Citrus sinensis* and *P. trifoliata* x *paradisi* (Koch *et al.* 1986). In the present study, this may have been an effect due solely to increased C availability. Results reported in Chapter 2 suggest that leaf size would remain unchanged by an exacerbated N limitation due to interaction with C supply (see Chapter 1). When Geiger *et al.* 1999 concluded that many plant responses to increased C supply can result from a nitrogen limitation due to interaction between N and C supplies, they indicated that photosynthetic acclimation and changes in leaf biochemistry may still be due partly to sugar related changes in gene expression. Perhaps there are also C effects on leaf morphology, that are unrelated to N limitation.

While leaves grown in elevated CO₂ concentration contained more nitrogen (Fig. 4.5), this can be accounted for by the greater leaf area and greater leaf mass (Fig. 4.3, Table 4.3). Nitrogen concentration (on a mass basis), N_m , was lower in leaves of trees grown at elevated CO₂ concentration (Fig. 4.4). This is consistent with the results of many studies (reviewed in Jarvis 1995; Wullschlegel, Post & King 1995; Chen, Impens & Ceulemans 1997; Poorter *et al.* 1997; Cotrufo, Ineson & Scott 1998; Saxe, Ellsworth & Heath 1998), and has been called “the dilution effect” (Overdiek 1990). It is now generally accepted that mass based nitrogen concentration in leaves and plant organs grown at elevated CO₂ concentration is lower than in growing at ambient concentration, and that it is independent of soil nitrogen supply (Ceulemans & Mousseau 1994). Cotrufo, Ineson & Scott (1998), using meta-analysis, reviewed data for 69 species and found C₃ woody plants grown at elevated carbon supply exhibited a reduction in N_m of 19%, similar to that shown

in Fig. 4.4 for *N. fusca*. It is important to note that Curtis (1996), also using meta-analysis, and reviewing data for 41 species of woody plants grown at elevated CO₂, observed that leaf nitrogen concentration was reduced only when expressed on a mass basis. The reduction was less, when N concentration was expressed on an area basis.

Leaf nitrogen concentrations on a mass basis fell between 1.8% and 2.4% for trees grown in ambient CO₂ concentration, and between 1.6% and 2.7% for trees grown in elevated CO₂ concentration (data not shown). This was within the range seen in younger trees grown at three concentrations of N supply (Chapter 2), and within the range reported for mature trees growing in the field (Adams, 1976). While leaf N concentration is used commonly as a guide to tree N status, the levels of key metabolites in N metabolism provide a better indicator of impending N limitation (Geiger *et al.* 1999), and may well have been lower in trees growing at elevated CO₂ concentration. The similarity in specific leaf area, *S*, (Table 4.3) must be accepted cautiously, and may be due to the mass of accumulated starch (Faria *et al.* 1996), or to an additional cell layer (Eamus & Jarvis, 1989; Evans & Lee, 1993). The effect of starch accumulation can be great. Sims *et al.* (1998) reported that an increase of 36% in the mass per unit area of leaves of *Glycine max* grown at elevated CO₂ concentration, was due simply to starch accumulation.

Root uptake of N commenced before budburst, in both treatments, similar to the pattern seen in younger trees (Chapter 3). This provided some evidence that growth at elevated CO₂ concentration did not defer the root uptake of N relative to any remobilisation of storage N (Fig. 4.5, Table 4.2).

In conclusion, juvenile trees of *N. fusca*, growing at elevated CO₂ concentration, for five years, exhibited no differences in the phenology of budburst

and commencement of leaf fall when compared with trees grown at ambient concentration. The absolute rate of leaf expansion was also unaffected, but the seasonal increase in the mass of individual leaves was delayed in trees growing at elevated CO₂ concentration. There was a trend for the number of old leaves to be held longer on trees growing at elevated CO₂ concentration, but measurement did not proceed beyond 75 days after the first leaf fall. Leaves of trees growing at elevated CO₂ concentration were greater in both mass and area. This greater leaf area was probably due to effects of enhanced C supply that were not related to an exacerbated N limitation. The increased mass may have been due to an accumulation of starch, or to an additional cell layer. Root uptake of N commenced prior to budburst, in trees of both treatments.

4.5 SUMMARY

It was established that root N-uptake in *N. fusca* starts before budburst, and continues throughout the process of expansion of new leaves. Remobilisation of storage-N for growth of new leaves during the same period was discussed in Chapter 3. Certain phenological events were unchanged by manipulating the C-supply under conditions of low N-supply. These included the timing of onset of budburst and leaf loss, and also the duration of budburst and the time taken for completion of leaf expansion. Other characteristics were changed. Bud mass at budburst was lower when C-supply was enhanced, and seasonal increase in bud mass was delayed. The area of fully-expanded leaves was greater. There was a marked trend for old leaves to be retained longer on trees after budburst. The implications of these results for an understanding of the phenology of *N. fusca*, in

relation to seasonal changes in environment and demand and supply of C and N for growth, are discussed in the next chapter.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

5.1 PRINCIPAL FINDINGS

The results of this study were consistent with the presence of a number of strategies for the seasonal acquisition and deployment of N and C for growth. A consideration of these allowed the phenology of *N. fusca* to be characterised and understood in terms of first, the inter-relationships between N and C demand, and resource availability and growth and secondly, the limitations and opportunities arising from the full range of environmental variation during each season. These are dealt with below.

5.1.1 A strategy for the evergreen habit in N. fusca

Phenological traits of evergreen habit (whole plant), and leaf longevity (the time period from individual leaf emergence to fall), have been explained in terms of maximising plant carbon gain (see Schulze, Kupperts & Matyssek, 1977 and Kikuzawa, 1995). In that context, Gower, Reich & Son (1993) concluded that trees supporting a lower foliage mass of short-lived photosynthetically efficient leaves can achieve a similar carbon balance to trees that support a large foliage mass of long-lived, photosynthetically inefficient foliage. However, studies of the response to manipulation of C supply, (see the Introduction to Chapter 4), suggest that tree growth in natural forests is unlikely to be carbon-limited because of low soil-N availability. Evergreen habit and lower rate of leaf production have also been described as a phenotypic response to moderate nutrient stress, becoming more evident in nutrient poor sites (Chapin, 1980; Chapin *et al.*, 1987). In general, while *N. fusca* inhabits more

fertile forest sites than other endemic species, and has higher nutrient requirements (Adams, 1976), these sites are still nutrient poor (Davis, 1990). Continued uptake of soil-N during winter, (reported in Chapter 3), and the retention of photosynthetically active leaves, also during winter, (reported in Chapter 2), support the conclusion that *N. fusca* maintains a canopy of leaves in order to scavenge soil N during winter dormancy.

5.1.2 A strategy for leaf loss to avoid drought stress, and a strategy for N storage that does not involve leaves

The site of N-storage in roots, and remobilisation to new growth, in spring, was unchanged by alteration of the N supply (Chapter 3). Results, reported in Chapter 2, supported the conclusion that *N. fusca* sheds leaves in order to avoid drought stress, and that this increases with soil N availability. Periods of below-average rainfall have been correlated with the onset of dieback in *Nothofagus* forests on both major islands of New Zealand, often at a whole stand level (see Ogden, Stewart & Allen, 1996). The distribution of this species along precipitation, elevation and soil nutrient gradients suggests that *N. fusca* competes best at more favourable sites (Chapter 1). However, during a lifetime of perhaps 450 years, a tree may endure severe drought every thirty years. A species that responds to favourable conditions with an increase in canopy size and leaf number, can be expected to have nitrogen storage and leaf loss strategies which facilitate survival during regular periods of intense drought. Reported studies have shown that nitrogen withdrawal from leaves shed in response to drought can be limited (e.g. Escudero & del Arco, 1987; del Arco, Escudero & Garrido, 1991). Leaves that might be shed regularly under these circumstances could be expected to have no role in strategies for internal N-storage, particularly in

a tree species that is sensitive to nutrient availability. In this context, root storage in *N. fusca* is significant. In the absence of drought induced canopy reduction, however, the photosynthetic activity of overwintering leaves (Chapter 2) could still support metabolism required for root N-uptake and storage.

5.1.3 A strategy for simultaneous root N uptake and remobilisation of stored N

Root N-storage has been associated previously with young winter-deciduous trees (e.g. *Malus domestica*, Taylor & May 1967; *Acer pseudoplatanus*, Millard & Proe 1991) and *Betula pendula* (Wendler & Millard 1996), but not with evergreen species (e.g. *Citrus* sp., Feigenbaum *et al.*, 1987; *Eucalyptus globulus*, Wendler *et al.*, 1995). Results reported in Chapter 3 showed that significant quantities of N were taken up into root storage, during winter, and that roots were the storage site from which N was remobilised into new growth in spring. Results, reported in Chapters 3 and 4, also support the conclusion that spring remobilisation coincides with root N-uptake from the soil. Simultaneous root N-uptake has been seen in the evergreen conifer *Picea sitchensis*, in contrast with winter-deciduous *Acer pseudoplatanus* where it was preceded by remobilisation of storage-N (Millard, 1994). However, evergreen conifers store N in leaves (see Millard, 1996), and the processes accompanying remobilisation of storage-N from roots, may be dissimilar for *N. fusca*. Either, remobilisation was associated with root senescence, or roots contained enough stored N to support root maintenance and N-uptake activity, and also metabolic processes involved in remobilisation. There was evidence in support of root turnover. In both HN and MN treatments, mean root dry mass declined (albeit insignificantly) during the remobilisation period (Fig. 3.2d). Also the quantities of labelled N found in new stems and leaves (Fig. 3.4a,c) were significantly lower than

the amounts remobilised from roots (Fig. 3.5c). There was, therefore, substantial loss of labelled N during remobilisation. Both events may have been due to root senescence. If root senescence accompanied N remobilisation from roots, then a number of associated phenological events required to be synchronised during the transition from old to new season's canopy. These would include, the loss of old leaves, (supporting a reduction in hydraulic demand for root water-uptake), budburst, reduction of root volume (due to senescence), growth of new roots, and new leaf expansion, (supporting an increase in hydraulic demand for water).

5.1.4 Phenology unchanged by altered C and N supplies

In New Zealand *Nothofagus* species, the onset of budburst is later at higher altitudes, and where variation in topography produces low temperatures (see Wardle, 1984). It may also be delayed in cloudy, wet districts compared with drier, sunnier districts at the same altitude (Wardle, 1963). However, changes in C or N supply did not alter the timing of budburst or leaf fall (Chapters 2,3 and 4), or the absolute rate of leaf expansion (Chapter 4), or the relationship between budburst and leaf expansion, and root N-uptake. These spring-early summer events are related to the triggering of peak demand for N and C at the start of the new growing season. This suggests that the strategies referred to above, are independent of changes in N or C supply.

5.1.5 Phenology that changed with altered N supply: a strategy for adjustment of canopy size in response to N supply.

Bud mass and numbers, and total leaf numbers, increased with N supply (Chapter 2). In contrast there was minimal change (if at all) in the photosynthetic capacities

or characteristics of individual leaves (apart from the possible influence of short-term imbalance between tree N status, and N supply, perhaps seen in the first cycle, Chapter 2). Therefore, the response of *N. fusca* to changes in N supply was essentially by an alteration of total leaf area. Consequently, increase in this resource for C uptake, in response to an increased N supply, was primarily by spatial enlargement of the whole canopy.

5.2 THE CONCEPTUAL MODEL

As a consequence of the experimental results reported in Chapters 2, 3 and 4, the model (Fig. 1.1), initially prepared to explain interactions between C and N supply and demand, based on remobilisation of storage-N from old leaves, required to be changed in two important respects (Fig. 5.1). First, the N-storage site was shown to be in roots (Chapter 3). Secondly, the points at which phenology might intervene in the processes can be identified. An explanation of Fig. 5.1 now follows:

- (i) In a comparison with Fig 1.1, it can be seen that certain essential features still remain. The components of N supply are the N pool and N remobilised from storage (the storage site now being the roots). Buds provide the basis for nutrient demand in spring, and the potential for new stem and leaf growth in the next season. As with Fig. 1.1, demands driven by stem cambial and root meristems are assumed to be subsidiary to those driven by bud meristems.

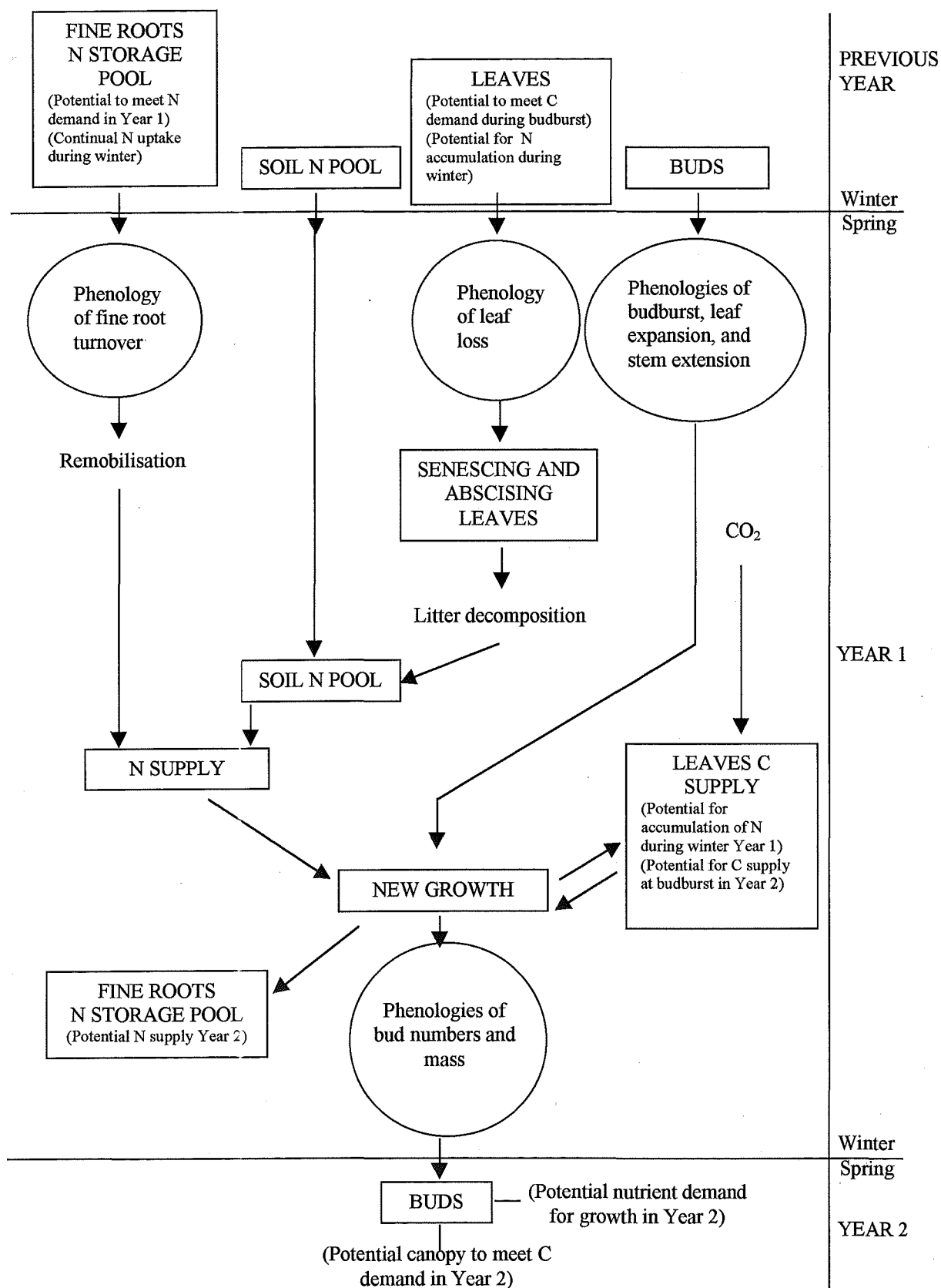


Figure 5.1 Diagram showing components of C and N supply, and interactions with C and N demand for growth in *N. fusca* for two successive seasons.

- (ii) The components of C supply are the canopy, and CO₂. The influence of supply and demand on growth in Year 1 is carried forward into Year 2, in the potential for demand and growth contained in the overwintering buds. The canopy of old leaves remaining in spring provides the basis for meeting the initial demand for C as spring growth commences with budburst, and until the new leaves become a sufficient source for photosynthate.
- (iii) The overwintering canopy of old leaves acts as a source of photosynthate required for root N-uptake during winter dormancy.
- (iv) Fine roots comprise the storage site for N, and root area is also one of the factors determining the quantity that may be taken up.
- (v) Nitrogen contained in falling leaves can only be a source of N supply through the processes of litter recycling and root N-uptake.
- (vi) Phenologies are indicated by circular margins. Those that define the commencement of events are budburst, leaf expansion, and stem extension. These determine the timing of onset of demand for C and nutrients, and of the start of new leaf function as a photosynthate source. Commencement of fine root turnover, similarly, determines the timing of N-availability from storage, root senescence (if present) and the commencement of new root growth. Those defining the cessation of events are leaf expansion and stem extension (timing of reduced demand for C and nutrients), and loss of old leaves (timing of functional end).
- (vii) If it is assumed that remobilisation of storage-N from roots is accompanied by root senescence, then all phenological events defining commencement of processes, and the loss of old leaves, must be synchronised in order to achieve the strategies suggested above for *N. fusca*.

5.3 ECOLOGICAL ISSUES

The results discussed in Chapters 2, 3 and 4, can be directly related to young and juvenile trees of *N. fusca* growing in the field. However, some care must be taken when extrapolating results achieved with young trees to mature trees, because of concerns that they may behave differently, both physiologically and phenologically (Eamus & Jarvis, 1989; Jarvis, 1995, Barton & Jarvis, 1999). It seems likely, nevertheless, that synchronisation of phenology in spring, linked to root storage of N, remobilisation into new seasonal stem and leaf growth and associated loss of old leaves, is also retained in mature trees. This is because the associated behaviours are at least as equally evident in mature trees. It has been observed that loss of old leaves, associated with spring budburst, becomes more enhanced as trees approach maturity, and that there is a single discrete flush of growth that is completed by early summer (Bussell, 1968).

Another ecological implication of the present findings may be in relation to possible responses to environmental change. There is potential for global increases in atmospheric CO₂ concentration to alter patterns of leaf senescence in some tree species (Jarvis, 1995). This alone is unlikely to alter processes of internal cycling of N in *N. fusca*, since the species relies on root storage. However, *N. fusca* must depend on litter recycling for recovery of N lost in falling leaves. Changes in the timing of senescence and leaf fall may affect that process. Furthermore, the synchronisation between root and shoot phenology in spring, presently suggested for *N. fusca* as part of the process of root N-storage, may be ecologically important in the context of poor soil N availability in natural forests, for several reasons. First, a

number of studies have shown that substantially greater root numbers, and root length, may result under elevated CO₂ concentration (Idso & Kimball, 1991, 1992; Korner & Arnone, 1992; Norby *et al.* 1992). It has also been predicted that optimum root length density is likely to increase under elevated atmospheric CO₂ concentrations (see van Noordwijk *et al.* 1998). Secondly, there may be changes in the spatial and temporal pattern of root development (Fitter *et al.* 1996), including some evidence (in *Populus x euramericana*) for reduced root longevity, and therefore increased turnover (Pretzinger *et al.*, 1995). It remains to be seen whether these changes might influence intra- or interspecific competition for soil resources, but it has been predicted that changes in plant strategies on root turnover may be expected if the likely period of water stress between rainfall events increases (van Noordwijk *et al.* 1998).

5.4 FURTHER STUDIES

A number of further studies, following on from the results reported in this thesis, can be suggested. These are discussed below:

5.4.1 Comparison with other species showing similar behaviour

A strategy of root N storage and remobilisation in spring that is temporally linked to root turnover, loss of old leaves, and budburst and expansion of new leaves, such as that seen in *N. fusca*, is arguably an adaptation to seasonal changes in two environmental variables. These are water availability and temperature. The cool-temperate and warm-temperate conditions of New Zealand produce occasional summer drought and also moderate winter conditions suitable for photosynthesis

and continued uptake of N during tree dormancy. The processes of spring-early summer deciduousness are readily contrasted with processes of winter-deciduousness, where the total absence of leaves during winter is preceded by withdrawal of N from leaves and retranslocation to overwintering storage sites within the plant, in autumn. It would be of interest to confirm that strategies shown in younger trees are also retained in mature trees of *N. fusca*. Similar spring-early summer loss of old leaves, and budburst and expansion of new leaves can be seen in *Quercus suber* (pers. obs.) and in other evergreen oaks e.g. *Q. rotundifolia* and *coccifera* (del Arco, Escudero & Garrido, 1991), and could form the basis for comparative study and analysis. Since N-retrieval from leaves shed in response to drought is poor, a strategy of root storage, similar to that seen in *N. fusca* might be expected in (non-leguminous) summer-deciduous trees that are found in savannas and tropical forests. Typically, these trees lose their leaves at the start of the dry season, or in drought, and then flower. Budburst occurs when drought conditions end (see Richards, 1996). A good example is *Brachychiton populneus* (Schott et Endl.) R. Br. (Sterculiaceae) which inhabits the “bottle tree” scrubs of Queensland (Richards 1996), and the related *S. acerifolius* (F. Muell.), both of which species are endemic to Australia (Boland *et al.* 1984).

5.4.2 Litter recycling

A strategy of leaf loss without maximisation of previous N-withdrawal from leaves, also places emphasis on the requirement for efficient retrieval of N lost from falling leaves through recycling of nutrients in litter. Pure *Nothofagus* stands cover large areas and, in these, the principal competitors for N contained in soil and litter are likely to be soil microbia. In particular, changes in the biochemistry of senescing

and falling leaves that may have evolved to confer a competitive advantage on the tree, should be investigated. Young trees of *N. fusca*, showed increases in the mass of moribund leaves, while mean specific leaf area, S , of the balance of total leaves was generally maintained at the same value (Chapter 2). It is known that total soluble carbohydrates increase in the overwintering leaves of the related species, *N. dombeyi*, and a correlation with frost-hardiness has been suggested (Alberdi *et al.*, 1989). The full range of sugars present was not characterised in that study. An increase in polysaccharides might precede the formation of substantial quantities of phenolics and tannins in senescing leaves (Hendry, 1993). The polyphenol concentration of decomposing litter has been implicated in controlling the proportion of mineral forms of N, (i.e. NH_4^+ and NO_3^-), relative to organic forms (e.g. protein-tannin complexes) in *Pinus muricata* communities (Northrup *et al.*, 1995). These substances are sparingly soluble, and recalcitrant to decomposition. This appeared to have the effect of facilitating N recovery through tree-ectomycorrhizal associations, due to ectomycorrhizal utilisation of protein-tannin complexes as a nitrogen source. At the same time, availability of N to competing organisms was minimised, and losses of N from leaching and denitrification, were reduced. (Northrup *et al.*, 1995). The *Nothofagus* genus, (as with the entire family, *Fagaceae*), shares a characteristic, and well documented (see Wardle, 1984), ectotrophic mycorrhizal association. It would not be unreasonable to expect the same process of pre-conditioning of leaves for N-recovery during recycling of nutrients in litter to be present in this species.

5.4.3 Determining the start and duration of remobilisation

The timing of remobilisation of storage-N, relative to root N-uptake, could not be defined effectively in the experiment reported in Chapter 2 because of the harvesting intervals. However, sample collections during the experiment reported in Chapter 4 were sufficiently frequent to show that root N-uptake occurred continuously during budburst and new leaf expansion. Where remobilisation of storage-N and root uptake proceed simultaneously, it is impossible to detect the relative proportions of storage (unlabelled) N and soil N, except by an analysis that distinguishes between, and identifies, labelled and unlabelled N-substances. Mass spectrometric analysis of N in xylem sap has been used to detect the transition in N dependence from storage to current year uptake in walnut trees (Deng, Weinbaum & De Jong, 1989), and more recently, in *Betula pendula* (Millard *et al.*, 1998). In both studies, storage-N was labelled with ^{15}N by applying labelling fertiliser in a previous season, so that remobilised N (in the first study), and amino acids (in the second study), could be characterised, in samples taken during the subsequent season. Millard *et al.* (1998) suggested that the information acquired from this analysis could be combined with sap flux measurements to enable quantification of remobilised N. This avoids the need to measure the total N content of all leaves in a mature canopy.

5.5 CONCLUDING STATEMENT

A question, posed at the start of this study, was:

Is it N supply, or is it C supply, which is important to phenological or physiological changes in the canopy (i.e. the plant organ responsible for C uptake) of N. fusca?

The results showed that N supply is responsible for adjustments in the size of the canopy, but that it produces no changes in the physiology of individual leaves. Neither N nor C supplies affected the timing of budburst or leaf loss, or the timing of processes of internal recycling of N in spring. These phenological events characterise the seasonal responses of *N. fusca*.

Importantly though, while this study focussed on the responses of this particular tree species to manipulations of nitrogen and carbon supply it highlighted the ecological importance of the acquisition and allocation of nutrients within time and space. These processes are important determinants of plant co-existence and competitive interactions. Huston & De Angelis (1994) point out that it cannot be assumed that the mixing of trees and the available pool of nutrients is either instantaneous or homogeneous. They have expressed the view that localised plant-environmental interactions can generate sufficient spatial heterogeneity to allow the local co-existence of competing individuals. It is important to recognise that heterogeneity may be temporal, as well as spatial. Synchronisation, and asynchronisation, of nutrient uptake are both important determinants of strategies that allow any particular tree to survive in a variable environment with seasonal changes, as an individual, and in competition with others. The mechanisms of synchrony are phenological. These define the periods of time within which the tree regulates its internal processes in relation to those that are external in the local environment.

ACKNOWLEDGEMENTS

The most important contributions made to this thesis are those of my supervisors, Drs Matthew Turnbull, David Whitehead and Peter Millard, whose help, advice and encouragement were inspirational. I thank them all.

John Byers and Graeme Rogers provided assistance in the construction and maintenance of the irrigation system. Alan Leckie provided assistance with the initial preparation of the open-top chambers at Bromley. Drs. Renate Wendler, Alistair Smith and Andy Midwood gave assistance with measurement of stable isotopic content and enrichment of samples. Nicole Lauren and Jackie Healey gave technical assistance, and wonderful support. I thank them all too.

Helpful discussion and encouragement has been provided at various times by many people, some of whom are listed here – Dr Alf Keys, Dr Renate Wendler, Dr Martin Parry, Dr William Laing, Dr David Tissue, Dr Kevin Griffin, Dr John Hunt, Dr Steve Thomas, Mr Tony McSeveny, Dr Peter Clinton, Associate Professor Tony Cole, Dr Laurie Greenfield, Dr John Walker, Dr Juliet Gerard, Dr Ashley Sparrow, Dr Jack Heinemann, Dr Adrian Walcroft, Professor Warwick Silvester, Dr Bruce Clarkson and Dr Avi Holzapfel. I am grateful to you all.

I gratefully acknowledge the funding provided from the Agricultural and Marketing Research and Development Trust and the University of Canterbury as Post-Graduate Scholarships. Additional funding was provided by the Foundation for Research and

Technology (contract number C09624), the University of Canterbury and the Scottish Executive Rural Affairs Department. The British Council Higher Education Links Scheme provided funds to support travel between New Zealand and the UK.

REFERENCES

- Adams, J.A. (1976) Nutrient requirements of four *Nothofagus* species in north Westland, New Zealand, as shown by foliar analysis. *New Zealand Journal of Botany* 14, 211-223.
- Alberdi, M., Meza-Basso, L., Fernandez, J., Rios, D. & Romero, M. (1989) Seasonal changes in carbohydrate content and frost resistance of leaves of *Nothofagus* species. *Phytochemistry* 28, 759-763.
- Barton, C.V.M. & Jarvis, P.G. (1999) Growth response of branches of *Picea sitchensis* to four years exposure to elevated atmospheric carbon dioxide concentration. *New Phytologist* 144, 233-243.
- Begon, M., Harper, J.L. & Townsend, C.R. (1990) Ecology: Individuals, Populations and Communities. Blackwell. Boston. p. 858.
- Bolland, D.J., Brooker, M.I.H., Chippendale, G.M., Hall, N., Hyland, B.P.M., Johnston, R.D., Kleinig, D.A. & Turner, J.D. (1984) *Forest Trees of Australia*. Melbourne, Australia : Thomas Nelson. pp 130-135, 637-641.
- Brix, H. (1981a) Effects of thinning and nitrogen fertilisation on branch and foliage production in Douglas fir. *Canadian Journal of Forestry Research* 11, 502-511.
- Brix, H. (1981b) Effects of nitrogen fertiliser source and application rates on foliar nitrogen concentration, photosynthesis, and growth of Douglas fir. *Canadian Journal of Forestry Research* 11, 775-780.
- Brix, H. & Ebell, L.F. (1969) Effects of nitrogen fertilisation on growth, leaf area, and photosynthesis rate in Douglas fir. *Forest Science* 15, 189-196.

- Bussell, W. T. (1968) The growth of some New Zealand trees. 1. Growth in natural conditions. *New Zealand Journal of Botany* 6, 63-75.
- Causton, D.R. (1977) A biologist's mathematics. Edward Arnold, London. p. 200
- Ceulemans, R. & Mousseau, M. (1994) Tansley Review No. 71: Effects of elevated atmospheric CO₂ on woody plants. *New Phytologist* 127, 425-446.
- Chapin, F.S. III. (1980) The mineral nutrition of wild plants. *Annual review of Ecology and Systematics* 11, 233-260.
- Chen, S.G., Impens, I. & Ceulemans, R. (1997) Modelling the effects of elevated atmospheric CO₂ on crown development, light interception and photosynthesis of poplar in open top chambers. *Global change biology* 3, 97-106.
- Comstock, J.P. & Ehleringer, J.R. (1992) Correlating genetic variation in carbon isotopic composition with complex climatic gradients. *Proceedings of the National Academy of Science* 89, 7747-7751.
- Cotrufo, M.F., Ineson, P. & Scott, A. (1998) Elevated CO₂ reduces the nitrogen concentration of plant tissues. *Global change biology* 4, 43-54.
- Coyne, P.I. & Van Cleve, K. (1977) Fertiliser induced morphological and chemical responses of a quaking aspen stand in interior Alaska. *Forestry Science* 23, 92-102.
- Curtis, P.S. (1996) A meta-analysis of leaf gas exchange and nitrogen in trees grown under elevated carbon dioxide. *Plant Cell and Environment* 19, 127-137.
- Curtis, P.S. & Wang, X. (1998) A meta-analysis of elevated CO₂ effects on woody plant mass, form and physiology. *Oecologia* 113, 299-313.
- DeJong, T.M. & Doyle, J.F. (1985) Seasonal relationships between leaf nitrogen content (photosynthetic capacity) and leaf canopy light exposure in peach (*Prunus persica*). 8, 701-706.

DeJong, T.M., Day, K.R. & Johnson, R.S. (1989) Partitioning of leaf nitrogen with respect to within canopy light exposure and nitrogen availability in peach (*Prunus persica*). *Trees* 3, 89-95.

Deng, X., Weinbaum, S. A. & DeJong, T. M. (1989) Use of labelled nitrogen to monitor transition in nitrogen dependence from storage to current-year uptake in mature walnut trees. *Trees* 3, 11-16.

Eamus, D. & Jarvis, P.G. (1989) The direct effects of increase in the global atmospheric CO₂ concentration on natural and commercial temperate trees and forests. *Advances in Ecological Research* 19, 1-55.

El Kohen, A., Venet, L. & Mousseau, M. (1993) Growth and photosynthesis of two deciduous forest tree species exposed to elevated carbon dioxide. *Functional Ecology* 7, 480-486.

Escudero, A. & del Arco, J.M. (1987) Ecological significance of the phenology of leaf abscission. *Oikos* 49, 11-14.

Evans, J.R. (1989) Photosynthesis and nitrogen relationships in leaves of C₃ plants. *Oecologia* 78, 9-19.

Evans, L. & Lee, H.S.J. (1993) The effect of elevated CO₂ on the long term growth of birch. *Journal of Experimental Botany* 44, (Supplement), 12.

Faria, T., Wilkins, D., Besford, R.T., Vaz, M., Pereira, J.S. & Chaves, M.M. (1996) Growth at elevated CO₂ leads to down-regulation of photosynthesis and altered response to high temperature in *Quercus suber* L. seedlings. *Journal of Experimental Botany* 47, 1755-1761.

Farquhar, G.D., Caemmerer, S von. & Berry J.A. (1980) A biochemical model of photosynthetic CO₂ assimilation in leaves of C₃ species. *Planta* 149, 78-90.

Farquhar, G.D. & Wong S.C. (1984) An empirical model of stomatal conductance. *Australian Journal of Plant Physiology* 11, 191-210.

Feigenbaum, S., Bielorai, H., Erner, Y. & Dasberg, S. (1987) The fate of ¹⁵N labelled nitrogen applied to mature citrus trees. *Plant and Soil* 97, 179-187.

- Ferrier, R.C. & Alexander, I.J. (1991) Internal redistribution of N in Sitka spruce seedlings with partly droughted root systems. *Forestry Science* 37, 860-870.
- Field, C. (1983) Allocating leaf nitrogen for the maximisation of carbon gain: Leaf age as a control on the allocation program. *Oecologia* 56, 341-347.
- Field, C. & Mooney, H.A. (1986) The photosynthesis-nitrogen relationship in wild plants. In: Givnish, T.J., ed. *On the economy and form and function*. Cambridge, UK: University Press, Cambridge, pp 25-55.
- Fife, D.N. & Nambiar, E.K.S. (1984) Movement of nutrients in Radiata pine needles in relation to the growth of shoots. *Annals of Botany* 54, 303-314.
- Fitter, A.H., Self, G.K., Wolfenden, J., van Vuuren, M.M.I., Brown, T.K., Williamson, L., Graves, J.D. & Robinson, D. (1996) Root production and mortality under elevated atmospheric carbon dioxide. *Plant and Soil* 187, 299-306.
- Fowler, J. & Cohen, L. (1992) *Practical Statistics for Field Biology*. John Wiley & Sons, Chichester, UK.
- Gaudilliere, J.P. & Mousseau, M. (1989) Short term effect of CO₂ enrichment on leaf development and gas exchange of young poplars (*Populus euroamericana* cv I 214). *Oecologia plantarum* 10, 95-105.
- Geiger, M., Haake, V., Ludevig, F., Sonnewald, U. & Stitt, M. (1999) The nitrate and ammonium nitrate supply have a major influence on the response of photosynthesis, carbon metabolism and growth to elevated carbon dioxide in tobacco. *Plant Cell & Environment* 22, 1177-1199.
- Gill D.S., Amthor, J.S. & Bormann, F.H. (1998) Leaf phenology, photosynthesis, and the persistence of saplings and shrubs in a mature northern hardwood forest. *Tree Physiology* 18, 280-289.

Grace, J.B. (1995) In search of the Holy Grail: explanations for the coexistence of plant species. *Tree* 10, 263-264.

Grant, P.J. (1984) Drought effects on high altitude forests, Ruahine Range, North Island, New Zealand. *New Zealand Journal of Botany* 22, 15-27.

Gunderson, C.A., Norby, R.J. & Wullschleger, S.D. (1993) Foliar gas exchange responses of two deciduous hardwoods during 3 years of growth in elevated CO₂: no loss of photosynthetic enhancement. *Plant Cell and Environment* 16, 797-807.

Hakkinen, R., Linkosala, T. & Hari, P. (1998) Effects of dormancy and environmental factors on timing of bud burst in *Betula pendula*. *Tree Physiology* 18, 707-712.

Heilman, P.E. & Xie, F-G. (1994) Effects of nitrogen fertilisation on leaf area, light interception and productivity of short rotation *Populus trichocarpa* x *Populus deltoides* hybrids. *Canadian Journal of Forestry Research* 24, 166-173.

Hendry, G.A.F. (1993) Plant pigments. In: Lea, P.J., Leegood, R.C. eds. *Plant biochemistry and molecular biology*. Chichester, UK: John Wiley & Sons Ltd, pp193-194.

Hogan, K.P., Whitehead, D., Kallarackal, J., Buwalda, J.G., Meekings, J. & Rogers, G.N.D. (1996) Photosynthetic activity of leaves of *Pinus radiata* and *Nothofagus fusca* after 1 year of growth at elevated CO₂. *Australian Journal of Plant Physiology* 23, 623-630.

Hunt, R. (1982) Plant growth curves. The functional approach to plant growth analysis. Edward Arnold, London, p248.

Huston, M.A. & De Angelis, D. L. (1994) Competition and coexistence: The effects of resource transport and supply rates. *The American Naturalist* 144, 954-977.

Ibrahim, L., Proe, M.F. & Cameron, A.D. (1997) Main effects of nitrogen supply and drought stress upon whole plant carbon allocation in poplar. *Canadian Journal of Forestry Research* 27, 1413-1419.

Idso, S.B. & Kimball, B.A. (1991) Effects of two and a half years of atmospheric CO₂ enrichment on the root density distribution of 3-year-old sour orange trees. *Agricultural and Forest Meteorology* 55, 345-349.

Idso, S.B., Kimball, B.A. & Allen, S.G. (1991) CO₂ enrichment of sour orange trees: 2-5 years into a long-term experiment. *Plant Cell and Environment* 14, 351-353.

Idso, S.B. & Kimball, B.A. (1992) Seasonal fine-root biomass development of sour orange trees grown in atmosphere of ambient and elevated CO₂ concentration. *Plant Cell and Environment* 15, 337-341.

Ingestad, T. & Lund, A-B. (1979) Nitrogen stress in birch seedlings. *Physiologia plantarum* 45, 137-148.

Jane, G.T. (1986) Wind damage as an ecological process in mountain beech forests of Canterbury, New Zealand. *New Zealand Journal of Ecology* 9, 25-39.

Jane, G.T. & Green, T.G.A. (1983) Episodic forest mortality in the Kaimai Ranges, North Island, New Zealand. *New Zealand Journal of Botany* 21, 21-31.

Jarvis, P.G. (1995) The role of temperate trees and forests in CO₂ fixation. In: Hirose T. & B.H. Walker (eds.) *Global change and terrestrial ecosystems in monsoon Asia*. Kluwer Academic Publishers, Belgium. pp 157-174.

Johnson, D.W., Ball, T. & Walker, T. (1995) Effects of elevated carbon dioxide on nutrient uptake in ponderosa pine seedlings. *Plant and Soil* 168, 535-545.

June, S.R. & Ogden, J. (1975) Studies on the vegetation of Mount Colenso, New Zealand 3. The population dynamics of red beech seedlings. *Proceedings of the New Zealand Ecological Society* 22, 61-66.

Kihachiro, K. (1995) The basis for variation in leaf longevity of plants. *Vegetatio* 117, 89-100.

Kirkland, A. (1961) Preliminary notes on seeding and seedlings in red and hard beech forests of North Westland and the silvicultural implications. *New Zealand Journal of Forestry* 8, 482-495.

Koch, K., Jones, P., Avigne, W. & Allen, L. (1986) Growth, dry matter partitioning and diurnal activities of RuBP carboxylase in citrus seedlings maintained at 2 levels of CO₂. *Physiologia plantarum* 67, 477-484.

Korner, C. & Arnone III J.A. (1992) Responses to elevated carbon dioxide in artificial tropical ecosystems. *Science* 257, 1672-1675.

Kuers, K. & Steinbeck, K. (1998) Leaf area dynamics in *Liquidambar styraciflua* saplings: responses to nitrogen fertilisation. *Canadian Journal of Forestry Research* 28, 1660-1670.

Kuppers, M. (1993) Carbon relations and competition between woody species in a Central European hedgerow. IV Growth, form and partitioning. *Oecologia* 66, 343-352.

Lee, H.S.J., Barton, C.V.M. & Jarvis, P.G. (1993) Effects of elevated CO₂ on mature Sitka spruce. *Vegetatio* 104/105, 456-457.

Legaz, F., Primo-Millo, E., Primo-Yufero, E., Gil, C. & Rubio, J.L. (1982) Nitrogen fertilisation in citrus. 1. Absorption and distribution of nitrogen in calamondin trees (*Citrus mitis* Bl.), during flowering, fruit set and initial fruit development periods. *Plant and Soil* 66, 339-351.

Legaz, F., Serna, M. D. & Primo-Millo, E. (1995) Mobilisation of the reserve N in citrus. *Plant and Soil* 173, 205-210.

Leegood, R.C. (1993) Carbon dioxide concentrating mechanisms. In: Lea PJ, Leegood, R.C. eds. *Plant biochemistry and molecular biology*. Chichester, UK: John Wiley & Sons Ltd, p. 69.

Leuning, R. (1997) Scaling to a common temperature improves the correlation between the photosynthesis parameters J_{\max} and V_{\max} . *Journal of Experimental Botany* 48, 345-347.

Maindonald, J.H. & Cox N.R. (1984) Use of statistical evidence in some recent issues of DSIR agricultural journals. *New Zealand Journal of Agricultural Research* 27, 597-610.

Marmann, P., Wendler, R., Millard, P. & Heilmeyer, H. (1997) Nitrogen storage and remobilisation in ash (*Fraxinus excelsior*) under field and laboratory conditions. *Trees* 11, 298-305.

Millard, P. (1996) Ecophysiology of the internal cycling of nitrogen for tree growth. *Z.Pflanzenernahr. Bodenk.* 159, 1-10.

Millard, P. & Nielsen, G.H. (1989) The influence of nitrogen supply on the uptake and remobilisation of stored N for the seasonal growth of apple trees. *Journal of Experimental Botany* 63, 301-309.

Millard, P. & Proe, M. F. (1991) Leaf demography and the seasonal internal cycling of nitrogen in sycamore (*Acer pseudoplatanus* L.) seedlings in relation to nitrogen supply. *New Phytologist* 117, 587-596.

Millard, P. & Proe, M. F. (1993) Nitrogen uptake, partitioning and internal cycling in *Picea sitchensis* (Bong.) Carr. as influenced by nitrogen supply. *New Phytologist* 125, 113-119.

Millard, P., Wendler, R., Hepburn, A. & Smith, A. (1998) Variations in the amino acid composition of xylem sap of *Betula pendula* Roth. Trees due to remobilisation of stored N in the spring. *Plant Cell and Environment* 21, 715-722.

Mitchell, H.I., Chandler, R.F. (1939) The nitrogen nutrition and growth of certain deciduous trees of the north-eastern United States. *Black Rock Forest Bulletin No. 11*.

Mousseau, M. & Enoch, Z.H. (1989) Carbon dioxide enrichment reduces shoot growth in sweet chestnut seedlings (*Castanea sativa* Mill). *Plant Cell and Environment* 12, 927-934.

Myeneni, R.B., Keeling, C.D., Tucker, C.J., Asrar, G. & Nemani, R.R. (1993) Increased plant growth in the northern high latitudes from 1981 to 1991. *Nature* 386, 698-702.

Nielsen, D., Millard, P., Nielsen, G.H. & Hogue, E. J. (1997) Sources of N for leaf growth in a high density apple (*Malus domestica*) orchard irrigated with ammonium nitrate solution. *Tree Physiology* 17, 733-739.

Norby, R.J., Gunderson, C.A., Wullschlegel, S.D., O'Neill, E.G. & McCracken, M.K. (1992) Productivity and compensatory response of yellow poplar trees in elevated CO₂. *Nature* 357, 322-324.

Norby, R.J., Pastor, J. & Mellilo, J. (1986) Carbon-nitrogen interactions in CO₂ enriched white oak: physiological and long-term perspectives. *Tree Physiology* 2, 233-241.

Northrup, R.N., Zengshou, Y., Dahlgren, R.A. & Vogt, K.A. (1995) Polyphenol control of nitrogen release from pine litter. *Nature* 377, 227-229.

Ogden J., Stewart G.H. & Allen R.B. (1996) Ecology of New Zealand *Nothofagus* forests. In:

Veblen, T.T., Hill, R.S., Read, J. eds. *The ecology and biogeography of Nothofagus forests*.

Michigan, USA : Yale Press, 25-82.

Overdieck, D. (1990) Effects of elevated CO₂ -concentration levels on nutrient contents of herbaceous and woody plants. In: Goodriaan, J., Van Keulen, H & H.H. Laar (eds.) *The greenhouse effect and primary productivity in European agro-ecosystems*. Wageningen, Pudoc pp. 31-37.

- Pallardy, S.G. & Rhoads, J.L. (1993) Morphological adaptations to drought in seedlings of deciduous angiosperms. *Canadian Journal of Forestry Research* 23, 1766-1774.
- Peace, W.J.H. & Grubb, P.J. (1982) Interaction of light and mineral nutrient supply in the growth of *Impatiens parviflora*. *New Phytologist* 90, 127-150.
- Pereira, J.S., Chaves, M.M., Fonseca, F., Araujo, M.C. & Torres, F. (1992) Photosynthetic capacity of leaves of *Eucalyptus globulus* (Labill.) growing in the field with different nutrient and water supplies. *Tree Physiologist* 11, 381-389.
- Poole, A.L. (1950) Studies of New Zealand *Nothofagus*. I. Taxonomy and floral morphology. *Transactions of the Royal Society of New Zealand* 78, 363-380.
- Pretzinger, K.S., Zak, D.R., Curtis, P.S., Kubiske, M.E., Teeri, J.A. & Vogel, C.S. (1995) Atmospheric CO₂, soil nitrogen and turnover of fine roots. *New Phytologist* 129, 579-585.
- Puntieri, J., Barthelemy, D., Martinez, P., Raffaele, E. & Brion, C. (1998) Annual – shoot growth and branching patterns in *Nothofagus dombeyi* (Fagaceae). *Canadian Journal of Botany* 76, 673-685.
- Persson, H. (1979) Fine root production, mortality and decomposition in forest ecosystems. *Vegetatio* 41, 101-107.
- Poole, A.L. (1987) *Southern Beeches*. Christchurch : New Zealand Department of Scientific and Industrial Research.
- Poorter, H., Van Berkel, Y., Baxter, R., Den Hertog, J., Dijkstra, P., Gifford, R.M., Griffin, K.L., Roumet, C., Roy, J. & Wong, S.C. (1997) The effect of elevated CO₂ on the composition and construction costs of leaves of 27 C₃ species. *Plant Cell and Environment* 20, 472-482.
- Radin, J.W. & Boyer, J.S. (1982) Control of leaf expansion by nitrogen nutrition in sunflower plants. *Plant Physiology* 69, 771-775.

- Read, J. & Farquhar, G. (1991) Comparative studies in *Nothofagus* (Fagaceae). 1. Leaf carbon isotope discrimination. *Functional ecology* 5, 684-695.
- Reich, P.B., Kloeppel, B.D., Ellsworth, D.S. & Walters, M.B. (1995) Different photosynthesis-nitrogen relations in deciduous hardwood and evergreen coniferous tree species. *Oecologia* 104, 24-30.
- Reich, P.B., Grigal, D.F., Aber, J.D. & Gower, S.T. (1997) Nitrogen mineralisation and productivity in 50 hardwood and conifer stands on diverse soils. *Ecology* 78, 335-347.
- Reich, P.B., Ellsworth, D.S. & Walters, M.B. (1998) Leaf structure (specific leaf area) modulates photosynthesis-nitrogen relations: evidence from within and across species and functional groups. *Functional ecology* 12, 948-958.
- Remphrey, W.R. & Davidson, C.G. (1994) Shoot preformation in clones of *Fraxinus pennsylvanica* in relation to site and year of bud formation. *Trees* 8, 126-131.
- Rogers, G.S., Milham, P.J., Thibaud, M.C. & Conroy, J.P. (1996) Interactions between rising carbon dioxide concentration and nitrogen supply in cotton I. Growth and leaf nitrogen concentration. *Australian Journal of Plant Physiology* 23, 119-125.
- Russell, R. S. (1936) The mechanism of leaf-fall in certain New Zealand trees. *Transactions and Proceedings of the Royal Society of New Zealand* 65, 407-421.
- Saxe, H., Ellsworth, D.S & Heath, J. (1998) Tansley review No. 98: Tree and forest functioning in an enriched CO₂ atmosphere. *New Phytologist* 139, 395-436.
- Setoguchi, H., Ono, M., Ono, Y., Yoshimichi, D., Koyama, H. & Tsuda, M. (1997) Molecular phylogeny of *Nothofagus* (Nothofagaceae) based on the atpB-rbcL intergenic spacer of the chloroplast DNA. *Journal of Plant Research* 110, 469-484.

- Schulze, E. -D., Kupperts, M. & Matyssek, R. (1986) The roles of carbon balance and branching patterns in the growth of woody species. In: T.J. Givnish, ed. *On the Economy of Plant Form and Function*. New York: Cambridge University Press.
- Silvola, J. & Ahlholm, U. (1993) Effects of CO₂ concentration and nutrient status on growth, growth rhythm and biomass partitioning in a willow, *Salix phylicifolia*. *Oikos* 67, 227-234.
- Sims, D.A., Luo, Y. & Seemann, J.R. (1998) Importance of leaf versus whole plant CO₂ environment for photosynthetic acclimation. *Plant Cell and Environment* 21, 1189-1196.
- Stitt, M. & Krapp, A. (1999) The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. *Plant Cell and Environment* 22, 583-621.
- Taylor, B. K. & May L. H. (1967) The nitrogen nutrition of the peach tree II. Storage and mobilisation of nitrogen in young trees. *Australian Journal of Biological Science* 20, 389-411.
- Thomas, R.C. (1987) Retention of ¹⁵N labelled urea in a radiata pine ecosystem: effect of split applications. *Ph.D. thesis*, University of Canterbury, Christchurch, New Zealand. 196 pp.
- Thomas, H. & Stoddart, J.L. (1980) Leaf Senescence. *Annual review of Plant Physiology* 31, 83-111.
- Tromp, J. (1983) Nutrient reserves in roots of fruit trees, in particular carbohydrates and nitrogen. *Plant and Soil* 71, 401-413.
- van Noordwijk, M., Martikainen, P., Bottner, P., Cuevas, E., Rouland, C. & Dhillon, S.S. (1998) Global change and root function. *Global change biology* 4, 759-772.
- van Steenis, C.G.G.J. (1953) Results of the Archbold expeditions Papuan *Nothofagus*. *Journal of the Arnold Arboretum* 34, 301-374.
- Veblen, T.T., Hill, R.S. & Read, J. (1996) Themes and concepts in the study of *Nothofagus* forests. In: Veblen, T.T., Hill, R.S., Read, J., eds. *The ecology and biogeography of Nothofagus forests*. Michigan: Yale University Press.

Walters, M.B., Reich, P.B. (1989) Responses of *Ulmus americana* seedlings to varying nitrogen and water status. 1. Photosynthesis and growth. *Tree Physiology* 5, 159-172.

Walters, M.B., Kruger E.L. & Reich P.B. (1993) Growth, biomass distribution and CO₂ exchange of northern hardwood seedlings in high and low light: relationships with successional status and shade tolerance. *Oecologia* 94, 7-16.

Walters, M.B. & Reich P.B. (1996) Are shade tolerance, survival, and growth linked? Low light and nitrogen effects on hardwood seedlings. *Ecology* 77, 841-853.

Walters, M.B. & Reich P.B. (1997) Growth of *Acer saccharum* seedlings in deeply shaded understories of northern Wisconsin: effects of nitrogen and water availability. *Canadian Journal of Forestry Research* 27, 237-247.

Waring, R.H., McDonald, A.J.S., Larsson, S., Ericsson, T., Wiren, A. & Arwidsson, E. (1985) Differences in chemical composition of plants grown at constant relative growth rates with stable mineral nutrition. *Oecologia* 66, 157-160.

Wardle, J. (1984) *The New Zealand Beeches: Ecology, Utilisation and Management*. New Zealand Forest Service.

Wardle, P. (1963) Growth habits of New Zealand sub-alpine shrubs and trees. *New Zealand Journal of Botany* 1, 18-47.

Weinbaum, S. A., Mervin, M.L. & Muraoka, T. T. (1978) Seasonal variation of nitrate uptake efficiency and distribution of absorbed nitrate in non-bearing prune trees. *Journal of the American Society of Horticultural Science* 103, 516-519.

Weinbaum, S. A., Klein, I., Broadbent, F. E., Micke, W. C. & Muraoka, T.T. (1984) Effects of time of nitrogen application and soil texture on the availability of isotopically labelled fertiliser nitrogen to

reproductive and vegetative growth of mature almond trees. *Journal of the American Society of Horticultural Science* 109, 339-343.

Weinbaum, S. A., Klein, I. & Muraoka, T. T. (1987) Use of nitrogen isotopes and a light-textured soil to assess annual contributions of nitrogen from soil and storage pools in mature almond trees. *Journal of the American Society of Horticultural Science* 112, 526-529.

Weinbaum, S. & van Kessel, C. (1998) Quantitative estimates of uptake and internal cycling of ^{14}N -labelled fertiliser in mature walnut trees. *Tree Physiology* 18, 795-801.

Wendler, R., Carvalho, P.O., Pereira, J. S. & Millard, P. (1995) Role of nitrogen remobilisation from old leaves for new leaf growth of *Eucalyptus globulus* seedlings. *Tree Physiology* 15, 679-683.

Wendler, R. & Millard, P. (1996) Impacts of water and nitrogen supplies on the physiology, leaf demography and nitrogen dynamics of *Betula pendula*. *Tree Physiology* 16, 153-159.

Whitehead, D., Hogan, K.P., Rogers, G.N.D., Byers, J.N., Hunt, J.E., McSeveny, T.M., Hollinger, D.Y., Dungan, R.J., Earl, W.B. & Bourke, M.P. (1995) Performance of large open-top chambers for long-term field investigations of tree response to elevated carbon dioxide concentration. *Journal of Biogeography* 22, 307-313.

Williams, D.G. & Ehleringer, J.R. (1996) Carbon isotope discrimination in three semi-arid woodland species along a monsoon gradient. *Oecologia* 106, 455-460.

Wong, S.C., Kreidemann, P.E. & Farquhar, G.D. (1992) CO_2 x nitrogen interaction on seedling growth of four species of eucalypt. *Australian Journal of Botany* 40, 457-472.

Wullschlegel, S.D., Post, W.M. & King, A.W. (1995) On the potential for a CO_2 fertilisation effect in forest trees. Estimates of a biotic growth factor based on 58 controlled-exposure studies. In: Wodwell A.W., Mackenzie, F.T., eds. *Biospheric feedbacks in the global climate system: Will warming feed the warming?* Oxford University Press, New York. pp 85-107.

APPENDIX

Photosynthesis-stomatal conductance model

The coupled photosynthesis-stomatal conductance model for individual leaves is described by Leuning (1995). The rate of photosynthesis, A , is given by Farquhar *et al.* (1980) as

$$A = \min \{A_c, A_q\} - R_d \quad (1)$$

where A_c and A_q are the assimilation rates limited by the RuBP (ribulose-1,5-bisphosphate) carboxylation activity of the enzyme rubisco (ribulose-1,5-bisphosphate carboxylase-oxygenase) and RuBP regeneration by the electron transport system, respectively, and $\min \{ \}$ refers to the minimum of the two rates. R_d is the daytime rate of respiration resulting from processes other than photorespiration.

The rate of assimilation limited by rubisco activity, A_c , is given by

$$A_c = V_{cmzx} \frac{c_i - \Gamma^*}{c_i + K_c [1 + (o_i / K_o)]} \quad (2)$$

where V_{cmzx} is the maximum rate of carboxylation activity by rubisco under conditions of saturating substrates of RuBP and CO_2 , c_i and o_i are the intercellular CO_2 and O_2 concentrations, Γ^* is the CO_2 compensation concentration in the absence of day respiration, and K_c and K_o are the Michaelis constants for CO_2 and O_2 ,

respectively. When the assimilation rate is limited by the regeneration of RuBP, A_q is given by

$$A_q = \frac{J_{\max} (c_i - \Gamma^*)}{4(c_i + 2\Gamma^*)} \quad (3)$$

where J_{\max} is the maximum electron transport rate at saturating irradiance.

The response of A_{\max} to irradiance, Q , is given by a non-rectangular hyperbola (Farquhar and Wong, 1984) as

$$\beta A^2 - (\alpha Q + A_{\max})A + \alpha Q A_{\max} = 0 \quad (4)$$

where A_{\max} is the maximum rate of photosynthesis at saturating irradiance, β defines the convexity of the hyperbola, and α is the quantum yield of electron transport.